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Controlling Structure and Properties in Biomatter via Physical Processing Pathways

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Abstract

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The increasing consumption of non-degradable plastics and their harmful effects on the environment urgently call for the design and fabrication of degradable and renewably sourced materials. Natural materials have been useful since ancient times for a variety of applications but were phased out in the last several decades in favor of petroleum-based polymers. Modern science, however, has shown that there is more to explore in the field of natural materials due to new understandings of the hierarchical structures and diverse molecules present in biomaterials. Due to concerns with plastic pollution, the effects of microplastics on human and ecological health, and a drive to reduce fossil fuel dependency, there is an increasing interest to harness the inherent properties of renewably-sourced biomaterials.

Algae are promising candidates for bio-based materials as they can grow in a wide variety of aquatic environments, are already cultivated at industrial production rates as food and supplements, and have a high molecular complexity. Typically, algal materials are

made from extracting specific molecules from the algae, converting those molecules into useful materials and letting the rest go to waste. Such methods destroy the hierarchical nano- and micro-structures that give biomaterials their inherent strength and toughness, and can generate chemical waste. Alternatively, making materials using the entire biological matter without extracting specific target components may allow for the retention of hierarchical interactions and structures and offers no-waste processing routes.

In this thesis, I investigate the possibility of using whole, non-extracted biomass to replace conventional plastic products, addressing the challenges inherent in biopolymer processing that distinguish them from conventional petrochemicals. I start by investigating the potential of creating cohesive bioplastics from unprocessed algal biomatter with equipment currently used in the petrochemical polymers industry (**Aim 1**). Then, I use a model system of polysaccharides to understand how colloidal processing influences structure and properties in the solid state (**Aim 2**). Finally, I use the findings from the previous two Aims to control the structures and properties of foams made from whole algal biomatter and present a case study focusing on the applicability of algal foams as acoustically absorptive devices (**Aim 3**).

In **Aim 1**, we analyze the mechanical and morphological differences of compression-molded bioplastics made from spirulina cells, scanning a range of temperature, pressure, and time conditions during fabrication, aiming to understand the structure-property relationships in this new material class. Three-point bending and compression are used to analyze the mechanical properties, showing that as temperature increases, strength and stiffness increase until a point at which the material starts degrading and reducing in strength again. Scanning electron microscopy (SEM) is used to analyze the morphological properties, which shows a transition from a packed-powder morphology at low temperatures and pressures to a homogenous matrix morphology at higher temperatures and pressures. An optimal flexural strength of 26 MPa occurs at a temperature of 140 °C and 7 kN of pressing force, which gives an impressive compression strength of 76 MPa. Our mechanical testing reveals that in the optimal processing conditions those bioplastics fall well within the commercial plastics range of performance. Fourier Transform Infrared (FTIR) spec-

troscopy is used in conjunction with X-Ray Photoelectron Spectroscopy (XPS) to analyze the changes in bonding induced by the manufacturing process. The results indicate that the compression molding process induces changes in protein conformation and hydrogen bonding to create a homogenous matrix. Pre-treatment steps and additives are used to modify the mechanical performance of the materials, and the biodegradability of the pure spirulina bioplastics in soil is assessed, showing an ability for these bioplastics to be home compostable.

In **Aim 2**, we design a binary biopolymer system that allows for studying the interactions between those components in the colloidal state, and subsequently, after lyophilization and characterization of the physical properties and structure of the resulting foams, reveals connections between colloidal processing and solid foam performance. The two key carbohydrates we select are representative of biopolymers found in algal and plant biomatter, cellulose and pectin. Green chemical treatments are used to modulate the electrokinetic interactions between those components. Zeta potential measurements show that treating bacterial cellulose with a citric acid-based deep eutectic solvent (DES) produces a more stable colloid without the use of harsh chemicals as is typically done. Small-angle X-ray scattering (SAXS) provides evidence that the DES treatment defibrillates the cellulose fibers, creating a denser network of fiber entanglements compared to non-treated cellulose. Rheological measurements show the effects of those network changes on the shear behavior of the colloids. The addition of pectin then further modifies the colloids, reducing the zeta potential and viscosity but improving the elasticity of the network. Foams are then created from the colloids by freeze drying, and mechanical testing and SEM are used to characterize the properties of the lyophilized colloids, which produce foams of entangled fibers. Adding DES moderately improves strength and stiffness while the addition of pectin completely changes the foam structure, reducing strength while improving the ability for the foams to recover strain.

In **Aim 3**, we provide insights into the processing-structure-property relationship that determines the performance of whole algal foams in acoustic insulation applications as a case study. FTIR and X-ray Fluorescence (XRF) show distinct differences in algae

composition, reflecting differences in species, growing condition, and processing conditions. Rheology is again used to characterize the algal colloids, showing that the differences in composition between the species contribute to differences in colloidal properties, with species higher in sulfur and starch creating both stiffer and more elastic colloids and gels than the other species. Sound absorption is measured via the two-microphone impedance tube method, showing excellent sound absorption at low to mid-range frequencies, with one species performing exceptionally well at higher frequencies. SEM and micro computed tomography (micro-CT) are used to assess the morphological differences between the foams and relate the morphologies to the sound absorption results. The CT data and acoustic data are combined using the Johnson-Champoux-Allard (JCA) model, which allows for standardized comparisons of the acoustic absorption performance of different materials as well as the extraction of viscous air flow properties within the foams. Next, flammability testing shows that the processing and therefore the composition of the algae contribute to some species immediately self-extinguishing after being removed from a flame, while some will burn and smolder. Finally, Aim 3 presents biodegradation data, with a discussion on why typical polymer biodegradation tests as well as traditional CT characterization techniques require new methods and approaches to getting reliable data when used on whole-biomatter materials.

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Judging by my fellow labmates, Dr. Roumeli seems to have an extremely good track record of picking great people to join her lab. Out of these labmates I first thank Drs. Andrew Jimenez and Paul Grandgeorge-Rognon, my postdocs during the first half of my Ph.D. Most of what I learned during my time in lab was from them. Whether it was polymer science, experiment and visual design, data analysis, managing my workload, and being a good mentor and teacher, I have these two to thank. I am extremely grateful that not only did I get to spend time learning from them in lab, I also became good friends with them outside of lab. They set the tone for the amazing lab culture we had, organizing hang-outs, dinners, bike rides, pick-up basketball games, dessert Mondays, and more, which were the highlights of my time in lab.

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1. Introduction

1.1. The Plastic Pollution Problem

The worldwide consumption of petrochemical-based plastics has been increasing alarmingly for decades. The total amount of plastic produced by 2050 is predicted to be 33 billion tons, compared to 0.28 billion tons in 2012¹. It is estimated that the cradle-to-grave life of plastics will account for approximately 10-13% of the global carbon budget by 2050². This reliance on petroleum for everyday materials leads to increased CO₂ emissions, contributing to the ongoing climate crisis.

At the end of life of a synthetic plastic product, improper classification, sorting, disposal strategies, and a lack of education on waste handling, leads to an overwhelming presence of plastics in the environment³. Global plastic pollution is expected to increase to over 100 million tons per year in 2050, compared to about 25 million tons in 2000^{4,5}. The chemical stability of common plastics makes them attractive for numerous applications, but is also responsible for slow degradation rates, which allow them to permeate the environment before fully degrading⁶. Of the 8.3 billion tonnes of plastics that have been produced since the 1950s, less than 10% has been recycled, about 12% has been incinerated, and almost 80% has been sent to landfills⁷. Once in landfills, the plastics are able to enter our natural environments over long periods of time, as they degrade due to UV radiation from the sun or other conditions that can arise from landfills. Plastic waste collects in landfills, waterways, and oceans, causing significant hazards to ecological and human health⁸⁻¹¹.

Even when properly disposed of, commodity plastics are most often incinerated, releasing carbon dioxide (CO₂) into the environment⁹. According to the US Environmental Protection Agency (EPA), the production of petroleum-based plastic packaging materials

in the USA reached 14.5 million tons in 2018, constituting 5% of municipal solid waste. Of this, only 13.6% was recycled, while 16.9% was incinerated for energy recovery. The remaining 69.5%, or 10 million tons, ended up in landfills¹². The combustion of petrochemical plastics rapidly transfers carbon from the slow (thousands of years) to fast (human lifetimes) carbon cycle¹³. To comply with the Conference of Parties agreement from 2015 (COP21) and limit the rise of global temperatures to 1.5 °C compared to pre-industrial levels, new materials must be developed with less harmful effects on the environment.

During the long degradation timeframes of these materials, plastics are fragmented into smaller pieces, termed microplastics, that can seep into food and water systems at all levels of the food chain, causing health hazards throughout the environment^{11,14,15}. The negative impacts of the wide distribution of plastic waste in our environment include trauma to animals upon ingestion as well as long-term health hazards such as increased cancer risk, potential neurotoxicity, and potential reproductive and developmental toxicity^{10,16–18}. Moreover, different forms of plastic waste present different challenges for those managing plastic disposal. For example, synthetic polymer foams such as expanded polystyrene (EPS), polyethylene (PE), and polyurethane (PU), provide inexpensive, lightweight, and versatile solutions for a variety of applications, ranging from protective parts for transportation and padding in shoes and seats, to food packaging and flotation devices. However, polymer foams are not recyclable, even if their bulk counterparts are. Polymer foam waste, especially from polystyrene, has become enough of a problem that several U.S. states and countries around the world have recently implemented bans on most polystyrene products^{19–21}.

Due to the increasing encroachment of wildfires into what is called the wildland-urban interface (WUI), as well as multiple high-profile fires caused by polymer foams, a relatively new area of study has emerged that focuses on the effects of plastics when burned. Plastics typically contain an array of additives for plasticization, flame retardancy, antioxidation, and UV stability²². These additives are prone to leaching, both when in the environment as microplastics as well as after burning to ash. While the complexity of polymeric materials and their additives makes it difficult to separate the effects of the polymer itself and the

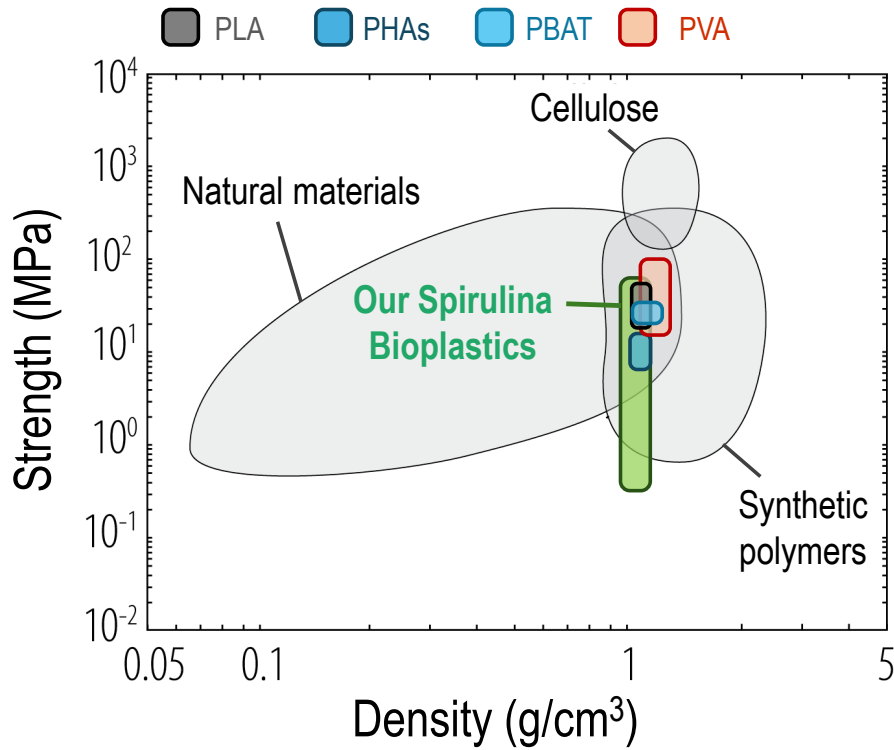


Figure 1.1: **Comparison of strength vs density of common bioplastics compared to spirulina bioplastics.**

additives inside it, recent studies highlight the increased toxicity that plastic products introduce to ash and the environment around burned areas²³⁻²⁵. Studies indicate that even new flame retardants seem to have similar toxicity to brominated flame retardants that were phased out due to their adverse health effects. Additionally, it appears that many flammability tests for things like furniture foams and household plastics are not representative of behavior in large flame scenarios such as those that would occur at WUIs. Therefore, Babrauskas et al. recommend rethinking the requirements to use these flame retardants by assessing the tradeoffs between fire hazards and flame retardant toxicity²⁶. Feuchter et al. used controlled chamber testing to assess the changes in smoke production and toxicity for various flame retardants in various polymer matrices. They concluded that, typically, multiple flame retardants are required for ideal performance in a variety of scenarios, but also that many flame retardants can have only mild or even negative impacts on smoke production and toxicity, emphasizing that polymeric materials must be carefully designed in conjunction with their flame retardants for adequate performance.

The alarming nature of this data has lead to increased interest in the development of sus-

tainable polymers, which includes macromolecules that are derived wholly or in part from renewable feedstocks and/or can biodegrade or be recycled⁸. To replace non-sustainable polymers, these new materials must meet the performance criteria of current polymers and must be able to be produced at a similar scale, which can be complicated based on the availability of feedstock, the cost of production, and the ability to be used with current plastic production infrastructure. For reference, the mechanical properties of commodity plastics usually range from 0.1 to 1 GPa in elastic modulus, between 1 and 50 MPa in strength, and from 1% to 1000% for elongation-to-break. A comparison of strength versus density for common bioplastics and the spirulina bioplastics from this thesis is shown in Figure 1.1. High-performance plastics have higher elastic moduli and strengths, 1-10 GPa and 30-200 MPa, respectively, but are more brittle, with elongation-to-break values below 10%¹.

1.1.1 Polymer foams for sound absorption

Besides bulk, thermoformable plastics, another type of plastic waste we specifically target in this thesis is acoustic foams, which are used to dampen room reverberations. Almost all polymer foams are made from materials in a liquid-like or melted state, in which gas bubbles are introduced either externally by gas injection, or internally by the use of blowing agents or through chemical reactions that produce gases. For example, polyurethane creates CO₂ bubbles due to interactions between isocyanate and water, while silicone-forming reactions release hydrogen gas. Creating bubbles via frothing or pressure-driven liquid-gas phase transition initiations are also potential externally-driven methods of foaming²⁷. Polymer foams can be made by extrusion, wherein a blowing agent or supercritical gas are homogenized with a polymer melt via shear from the extruder screws. Depending on the settings of the extruder, such as the screw speed and the variation of temperature along the length of the barrel, foams can be formed through the nucleation, growth, and stabilization of bubbles in the polymer matrix. Alternatively, forms can form via a pressure release mechanism at the end of the extruder, where upon leaving the pressurized extruder barrel, the gas bubbles expand within the polymer matrix^{28,29}.

A common method of foam production is expansion, such as what is used for expanded polystyrene (EPS). The blowing agent is often n-pentane, which boils as the polymer beads are heated by steam, causing expansion of the beads and the creation of a closed-cell foam structure. These expanded beads can then be packed into a mold and steamed again for further expansion to the point that it completely fills the mold, after which the product is finished³⁰. Cell sizes can depend on density of the foam, temperature at which it was processed, and more, but cell sizes typically range from 50 to 150 μm ³¹. However, a relatively unexplored method of foam production, solid-state foaming, can produce cell sizes of around 10 μm . This process comprises of putting a plastic piece inside a pressurized chamber with CO_2 or N_2 gas to saturate the gas within the polymer matrix. Once saturation has been achieved, the pieces are removed from the pressure chamber and placed into a hot liquid bath, putting the plastic at a temperature above its glass transition temperature. The material softens and the bubbles expand to create a porous matrix, typically with a dense skin layer covering the matrix³².

Polyurethane foams, which account for at least 50% of polyurethane consumption, are made by first mixing an isocyanate with a polyol and water. Most of the isocyanate reacts with the polyol to form urethane bonds, but some amount reacts with water, creating carbon dioxide gas and an unstable carbamic acid. The carbon dioxide acts as the blowing agent, while the carbamic acid reacts with isocyanates to form polyurea, which acts as a separate “hard segment” phase within the foam³³. The ratio of hard segments to polyurethane (soft segments) can be used to tune foam properties³⁴. For all of the benefits of polyurethane foams, they are also made from toxic ingredients that can pose significant health hazards to humans and the ecosystem. Isocyanates especially have high toxicity when not reacted with other chemicals, posing risks to those who produce the foams. Additionally, unreacted isocyanates are health risks after production. Aliphatic and aromatic amines, which can be used as catalysts for polyurethane production, are potentially carcinogenic³⁵.

Most acoustic absorption panels are made from polymer foams, often encased in a frame and covered with fabric for both aesthetic and functional purposes. Cross-linking

poly(vinyl alcohol) foams with formaldehyde can create poly(vinyl formal) gels that can then be freeze-dried to make sound absorption foams. Modifying the formaldehyde amount affects the pore sizes and shapes, giving pore sizes of 10 μm at the lowest formaldehyde content and 3 μm at the highest formaldehyde content. These foams have slow increases in sound absorption coefficient at low frequencies, but reach their maximum sound absorption at around 2000 Hz³⁶. Polyimide foams are made with CO₂ gas as the blowing agent, formed from reactions between water and isocyanates. This created closed-cell foams with cell sizes on the order of 1000 to 2000 μm . Foams made with no 4,4'-diaminodiphenyl ether (ODA) exhibited excellent sound absorption after approximately 1500 Hz, but increasing the ODA amount greatly reduced acoustic absorption capabilities³⁷. However, for reasons including cost, resistance to environmental factors, consistency in properties, control over macro- and micro-morphologies, and excellent mechanical properties, polyurethane and polyester foams, as well as polyester fiber mats, are commonly-used materials for sound absorption panels^{38–40}.

Polyurethane panels are especially often put up on walls in professional and amateur recording studios, lecture halls and classrooms, and clubs and dance halls. Polyurethanes are inherently highly flammable due to their chemical structure, and their ubiquity with a lack of protection from fire can lead to high heat release events and rapid flashover⁴¹. Cheap polyurethane panels can sometimes include toxic anti-flammability components to reduce fire risks^{42–44}. While regulations are starting to require the usage of safer materials, such restrictions are not strongly enforced and are easily circumvented. Additionally, the time required to study substitute flame retardants allows for the unsafe use of toxic materials in the meantime. Halogenated flame retardants, such as benzoic acid, 2,3,4,5-tetrabromo-, 2-ethylhexyl ester (TBB) and di(2-ethylhexyl) tetrabromophthalate (TBPH) all pose at least moderate toxicity hazards for humans, aquatic environments, and environmental persistence/bioaccumulation. Chlorinated phosphorous flame retardants, which can be seen as alternatives to halogenated additives, generally have high aquatic, carcinogenic, reproductive, and developmental toxicity. Inorganic, non-halogenated flame retardants such as ammonium polyphosphate (APP) and expandable graphite pose significantly lower health

and aquatic environment hazards, but very high environmental persistence. Melamine, a popular material used in interiors of commercial buildings and another inorganic, non-halogenated flame retardant, has generally moderately high toxicity for humans, including high reproductive toxicity. Finally, phosphate alternatives such as triphenyl phosphate (TPP), tricresyl phosphate (TCP), and Tris (p-t-butylphenyl) phosphate (TBPP) have a range of health hazards for humans, with TPP generally being the safest, although it is still highly hazardous to aquatic environments⁴⁵. Even if polymer foams can resist flammability, heat can still cause them to melt, which can allow for the transfer of heat to other, more flammable parts of a building. Therefore, there remains an urgent need for cheap, performant acoustic absorption materials that are non-petroleum derived, biodegradable, and flame-resistant.

1.2. Current Sustainable Solutions

1.2.1 General Classification

There are 4 broad categories of polymers when it comes to their sourcing and degradability, as shown in Figure 1.2. Petrochemical-based, non-biodegradable plastics such as polyethylene or PVC are the most common plastics used today. These contribute to the depletion of a non-renewable resource, petroleum, for its production, as well as increasing the amount of microplastics in our environment due to its non-degradable nature. Some plastics are biodegradable but are still sourced from petroleum. These materials are relatively expensive but are increasingly common in use these days.

Bio-based versions of conventional polymers exist as non-petroleum-derived alternatives, but they are not biodegradable, just like their petroleum-based counterparts. Finally, bio-based and bio-degradable polymers are relatively new and expensive, although some, such as poly(lactic acid), are increasingly common.

Petrochemically-derived biodegradable polymers

Petroleum-derived, biodegradable polymers such as polybutylene adipate terephthalate (PBAT) provide useful materials that can degrade in soil. PBAT in particular has become

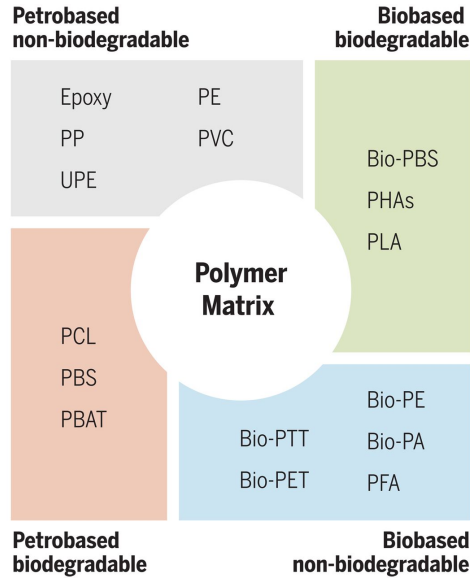


Figure 1.2: **Polymer sourcing and degradability.** Sourced from⁸.

very popular as a biodegradable plastic film, especially in conjunction with other plastics or biopolymers such as thermoplastic starch (TPS). It is created via poly-condensation between butanediol, adipic acid, and terephthalic acid, with organometallic compounds as catalysts, and can be created using conventional polyester manufacturing equipment. Polymerization requires high temperatures (above 190 °C), high vacuum, and long reaction times in order to remove the water created by the condensation reaction. Long chain branching PBAT can be created using branching agents such as isocyanates, alcohols, and acids, introducing branching into the polymer backbone and improving the melt strength. This allows for the production of stable, thin films with enhanced strength, using conventional polymer processing techniques, which is important for lowering the cost of the material while satisfying the needs of the products that use it⁴⁶. Additionally, it can be compounded with starch or PLA to greatly improve its usefulness. PBAT has a moderate tensile strength of 21-36 MPa, low flexural strength of 7.5 MPa, and a low Young's modulus of 20-35 MPa. However, its key advantage as a polymer lies in its impressive elongation to break of 650-700%. It is a soft polymer with a glass transition temperature of -30 °C and melts at a useful processing temperature range of 115-125 °C. Terephthalate content has a significant effect on the biodegradability of PBAT, with microbial enzymatic activity and hydrolysis dominating degradation behavior. Chain scission is typically favored at

the chain ends due to the higher free volume compared to the middle of the chain. However, once the molecular weight distribution narrows, main chain scission starts to occur in the middle of the chains and the shift in the molecular weight distribution changes much faster. PBAT can degrade in manure, food, and yard compost, as well as in both aerobic and anaerobic conditions. The hydrolytic cleavage of ester groups allow microorganisms to degrade the polymer, and cocrystallized regions of the adipate and terephthalate portions of the polymer tend to degrade more slowly than the amorphous regions, which is expected^{46–48}.

Polyvinyl alcohol (PVA) is another polymer that is often synthesized from petroleum-based sources but is also biodegradable. It is often obtained as a resin by the hydrolysis of polyvinyl acetate, where part of the alcoholysis step creates PVA. The properties of PVA can be modified based on its extent of hydrolysis, where partially hydrolyzed PVA will have some amount of polyvinyl acetate in it. PVA is soluble in water and can be crosslinked into hydrogels with boric acid, glutaraldehyde, formaldehyde, sodium nitrate, or UV light. This, along with its biocompatibility, makes it an attractive polymer for a variety of applications such as hygiene products, drug delivery, wound dressings, and contact lenses^{49,50}. However, PVA’s affinity for water can also be detrimental to some use cases, as it can swell undesirably when in humid or wet environments, so chemical modifications have been explored to enhance its water resistance. At low concentrations (2.5% in water) PVA exhibits shear thinning behavior, but as concentration increases to 5% or higher, it changes to Newtonian or semi-Newtonian behavior⁵¹. Dry PVA films show brittle failure with a strain at break of 25% with tensile strength of around 100 MPa and elastic modulus of about 2.2 GPa. As water is added, even at low concentrations, the film exhibits ductile failure, with initial failure leading to a plateau, indicating yielding as polymer chains rupture and rearrange. At 1.8% water, the tensile strength drops by about 30%, and further increases in water decrease the strength more while improving ductility⁵². The addition of nano-fillers such as silver or titanium nanoparticles and nanocellulose have been used to alter the crystallinity and hydrogen bonding of the polymer matrix⁵³. PVA is generally made up of repeating diol units, which are susceptible to both oxidation and

hydrolysis. *Pseudomonas* sp. strain VM15C has been shown to use a PVA dehydrogenase to oxidize PVA into β -diketone structures, which can then be hydrolyzed by oxidized PVA hydrolase⁵⁴.

Poly(butylene succinate) (PBS) was first developed in Japan from fossil fuel sources, but has since been produced commercially with 50% biobased content derived from fermentation products of starches, cellulose, and glycerol. PBS is an aliphatic polyester comprised of 1,4-butanediol and succinic acid, both of which are typically synthesized from petroleum feedstock, although succinic acid can now reliably be synthesized via fermentation. 1,4-butanediol can then be synthesized by the catalytic reduction of succinic acid, although a 100% biobased PBS method doesn't appear to be commercially viable yet. The first synthesis step can consist of either the esterification of 1,4-butanediol and succinic acid or the transesterification of 1,4-butanediol and dimethyl succinate. This forms PBS oligomers which can then undergo polycondensation with the removal of the 1,4-butanediol⁵⁵. Typically, the melting point is at around 115 °C, making it easy to process in conventional polymer processing equipment. It exhibits ductile behavior with an elongation to break of over 300%, a tensile yield strength of around 30-35 MPa, and a Young's modulus of about 3 GPa, depending on the crystallinity of the polymer. PBS is biodegradable in both soil and composting environments, although it degrades faster in compost environments with higher temperature, humidity, and favorable microbes. Microorganisms degrade PBS by hydrolysis of the ester linkages in the PBS backbone. As expected, biodegradation typically initiates at the amorphous regions. PBS is readily biodegradable in aerobic environments, but requires pre-treatment to degrade in anaerobic environments⁵⁶. Alkaline treatment with NaOH helps to reduce hydrophobicity of the polymer, which increases the accessibility of microbes to the bioplastic thus enabling faster hydrolysis.

Poly(ϵ -caprolactone) (PCL), which was first synthesized in the 1930s, is a has become popular as a tissue scaffolding material due to its desirable viscoelastic properties and its resorbability. PCL is an aliphatic polyester typically synthesized by one of two methods. Polycondensation of 6-hydroxyhexanoic acid can be done without a catalyst, but leads to molecular weights less than 10 kDa and is therefore not favored. Instead, ring-

opening polymerization of ϵ -caprolactone catalyzed by metal-based catalysts such as metal alkoxides or carboxylates is generally preferred. A downside to this approach is that elevated temperatures of 120 °C or more are required, and metal-based catalysts are toxic and require removal from the polymer post-polymerization. Research into magnesium and calcium-based catalysts have shown promise for producing low polydispersity polymers with low toxicity. Molecular weights typically range from 50.4 to 124 kDa by weight average, and melting temperatures are quite low, in the range of 56-65 °C⁵⁷. Tensile strengths can be up to 42 MPa, with highly ductile failure at strains of up to 1000% and Young’s modulus of up to 440 MPa. PCL has a glass transition temperature of -60 °C, making it rubbery at room temperature^{58,59}. The main degradation mode of PCL is random chain scission driven by the hydrolysis of ester bonds. Hydroxy end groups undergo oxidation at elevated temperatures of 120 °C, degrading into hydroperoxide radicals, leading to chain unzipping and a decrease in the molecular weight distribution. As with most polymers, the amorphous regions degrade more readily than the crystalline regions⁶⁰. PCL readily undergoes pH-mediate degradation with both base and acid catalysts, and animal studies have shown that it is bioresorbable and passes safely through the body, with no evidence of accumulation in organs or tissues⁶¹. This makes them highly desirable for use in the biomedical field, but PCL is also fully biodegradable by bacteria and fungi, and therefore has increased interest in materials outside of biological applications⁶².

PBAT, PVA, PBS, and PCL represent steps required to solve the biodegradation issue that comes with traditional synthetic plastics, but they are mostly sourced from petroleum products, making them non-renewable polymers. Instead, bio-based and biodegradable polymers have also been widely explored.

Natural and biodegradable polymers

Naturally derived polymers can be produced from bacteria, plants, and even animals, but their biodegradability is not assured despite the non-petroleum source. An overview of some different strategies for bioplastic production is shown in Figure 1.3.

Polylactic acid (PLA), the largest production volume alternative in this category, is produced by the fermentation of plant-based biomass such as corn or other similarly starchy

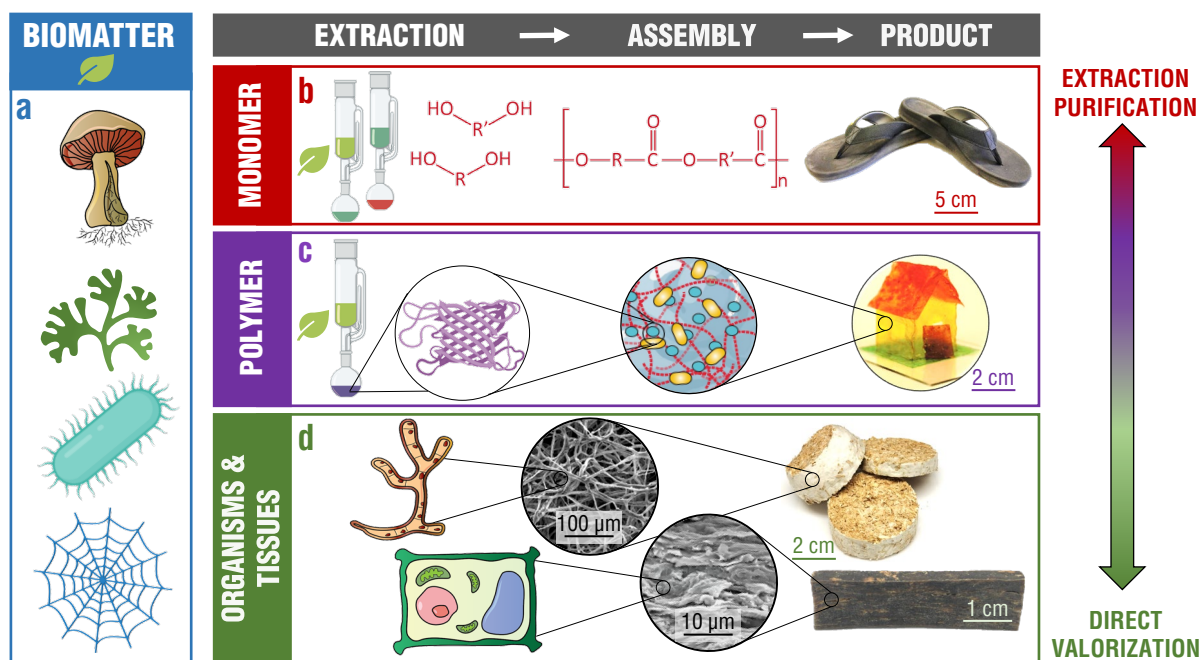


Figure 1.3: **Strategies for bioplastic production.** Research tracks to produce sustainable materials from renewable sources. (a) Examples of biomatter resources. (b) Extracting monomers to synthesize existing or entirely novel polymers. (c) Extracting biopolymers to create self-bonded or composite materials. (d) Using the entire organism as a polymer composite building block. Sourced from Campbell and Lin et al.¹.

plants and the ring-opening polymerization of the resulting lactic acid monomer¹. While there are a multitude of polymerization pathways for PLA, two are used significantly more often than the others. Both of these start with the fermentation of glucose to produce lactic acid, after which a catalyst and elevated temperatures (100-140 °C) are used to produce PLA oligomers. From here, one method is to directly polymerize the oligomers into polymers via a dehydration-condensation reaction at temperatures of 130-180 °C with the help of a catalyst. The other method, which is more common due to its higher efficiency, uses ring-opening polymerization at 200-230 °C with a catalyst to produce lactide, which can then be converted into polylactic acid at reduced temperatures of 80-110 °C. These methods can produce three different stereoisomers of PLA, depending on the orientation of the asymmetric CH₃ side group^{63,64}. For example, if L-lactide is used to make PLA, the resultant polymer would be poly (L-lactide) (PLLA), but if D-lactide is used, the output would be poly (D-lactide) (PDLA). If both are used, the result is a racemic mixture in the form of poly (DL-lactide) (PDLA)⁶⁵. PLA films can have tensile strengths ranging from

14 to 70 MPa depending on the molecular weight and relative L-lactide versus D-lactide content⁶⁴. They are relatively brittle, with elongation to break of 1 to 8%. PLA's rigidity, while good for some applications, can make it undesirable for many applications in which plastics are commonly used. Therefore, PLA can often be blended with other plastics to improve its mechanical properties. PLA also has poor barrier properties, which makes it a poor choice for packaging of things such as food or other things that are sensitive to their environment. While PLA is compostable, it can only be composted in specific environmental conditions of temperatures over 58 °C and with selected microbes, for which industrial facilities are required but not available to most communities⁶⁶. Degradation is driven by the hydrolysis of ester bonds, which break into carboxylic acid and alcohol, reducing the polymer chains into oligomers that can be consumed by microbes. PLA degradation mainly occurs through the scission of ester bonds and is induced by several factors, such as oxidation, photodegradation, thermolysis, hydrolysis, and enzymolysis. In natural soil, the degradation of PLA is very slow due to the low number of fungal and bacterial strains that naturally degrade the polymer⁶³.

Biobased and biodegradable polymers, such as poly(hydroxyalkanoate)s (PHAs), have the potential to reduce petroleum dependency and plastic pollution. While PHAs can be synthesized through the ring opening polymerization of lactones and cyclic diesters, but they can also be synthesized for energy storage within some types of bacteria, typically accumulating intracellularly under adverse growth conditions^{67,68}. Rather than being a specific polymer, PHAs are a family of polymers that includes over 150 unique monomers, the variety of which contribute to a broad spectrum of properties⁶⁹. Melting points can range from 50 to 180 °C and crystallinities can range from 30% to 70%^{70,71}. Short-chain PHAs can have between 3 to 5 carbons per repeat unit while medium-chain PHAs can have 6 to 14 carbons⁷². These two classes of PHAs are synthesized via different pathways by different types of microorganisms. Short-chain PHAs are synthesized by many types of bacteria and archaea by a three step process. First is the condensation reaction of acetyl-CoA or propionyl-CoA via β -ketothiolase. Next, this is reduced to (R)-3-hydroxyacyl-CoA, which is then followed by polymerization via a PHA synthase, PhaC, which is geneti-

cally encoded in these microorganisms by the *phaA-phaB-phaC* operon. Medium-length PHAs are typically synthesized by *Pseudomonas* species and related microorganisms. Enzymes such as enoyl-CoA hydratase (PhaJ) and (R)-3-hydroxyacyl-ACP:CoA transacylase (PhaG) divert enoyl-CoA and (R)-3-hydroxyacyl-ACP into the PHA biosynthesis pathway to further polymerize PHA. The synthesis and properties of PHAs can be modified in-situ by genetic engineering and feedstock changes for the synthesizing bacteria and ex-situ by chemical modifications^{68,72}. Molecular weights can range from 200 to 3,000 kDa, and homopolymeric PHAs will typically have higher crystallinities (50-90%), melting temperatures (approaching 170 °C), and glass transition temperatures (around 5 °C). These materials are often brittle, with elongation to break of less than 10%, so introducing comonomers such as 3-hydroxyvalerate can be used to toughen the materials. The disruption of crystalline packing by these comonomers can reduce the melting temperature (120 °C) and increase elongation to break to over 400%⁷³⁻⁷⁵. By taking advantage of biosynthesis pathways, PHAs can be functionalized in-situ for enhanced functionality. For example, azide-bearing copolymers were produced by introducing ω -azido-fatty acids, which created rubbery PHAs with pendant azide groups and glass transition temperatures of -45 °C⁷⁶. Another example is the synthesis of poly[(R)-3-hydroxybutyrate-*co*-(R)-3-hydroxy-10-undecenoate] (PHBU), which could be crosslinked for elongation to break greater than 450%, tensile strengths of 20 MPa, and a low melting temperature of 27 °C. PHAs are biodegradable under both aerobic and anaerobic conditions via enzymatic hydrolysis and thermolysis⁷⁷. Low molecular weight PHAs are generally more susceptible to biodegradation, and highly crystalline PHAs are harder to degrade than more amorphous PHAs. Many bacteria are able to degrade PHAs, including most of the bacteria that produce them. The intracellular degradation pathway involves a PHA depolymerase that breaks down, for example, poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(3HB)], into 3-hydroxybutyric acid. A dehydrogenase then oxidizes this acid into acetylacetate, before breaking down into acetyl-CoA via a β -ketothiolase, which is present in both the biosynthesis and biodegradation pathways. The specifics of the enzymes are difficult to study due to the complexity of the process and the close link between the polymer and the depoly-

merases. *In vivo*, biodegradation of the amorphous phases occurs first, potentially over the course of a few weeks. After this, erosion processes lead to a reduction in polymer mass of the mono-, di-, and trimers, and it can take another 2-3 years to degrade fully⁷⁸.

Waste valorization

Another solution to the plastic pollution issue is the valorization of plastic waste. Valorization is the process of recovering materials and energy from plastics that would otherwise be recycled or landfilled, specifically with the goal of increasing the value of the created product. This converts a plastic product’s linear lifetime into a circular lifecycle, minimizing waste and potentially reducing the plastic industry’s reliance on fossil fuels⁷⁹. For example, plastic can be used to create hydrogen gas via pyrolysis, photocatalysis, and electrocatalysis. Pyrolysis is the most commonly used method, but is also the dirtiest, producing CO₂ and requiring a large amount of energy. Photocatalysis and electrocatalysis methods are an emergent method that, while promising, need further study⁸⁰. Methods for converting plastics into carbon nanotubes, activated carbon materials, and fuels are also under study, but have similar drawbacks to the pyrolysis method of converting polymers to hydrogen gas. Finally, most plastic waste valorization methods require expensive ruthenium or platinum-based catalysts, which are expensive and reduce the promise of using these methods industrially. Recent efforts to replace these catalysts with more common earth abundant metals such as cobalt and nickel have shown promise as cost-effective methods for the cleavage of C-C bonds in polyolefins⁸¹. More recently, methanolysis, alcoholysis, and hydrogenolysis have been explored as ways of depolymerizing plastic waste for upcycled use cases. For example, poly(pentylene adipate-co-terephthalate) (PPeAT60) was combined with methanol and a dimethylethylamine (DMEA) catalyst to recover 1,5-pentanediol (PDO), dimethyl adipate (DMA), and dimethyl terephthalate (DMT), which all make up the polymer backbone. These were then able to be repolymerized into PPeAT with almost identical chemical properties and slight variations in mechanical properties⁸². Nylon was depolymerized into ϵ -caprolactum via acid-catalyzed alcoholysis, yielding significant techno-economic and environmental improvements in the life cycle of nylon 6 production, and the same was shown for the hydrogenolysis of polyethylene into linear and

branched hydrocarbons and light olefins, depending on the catalyst choice^{83,84}. Polyolefins have been especially difficult to upcycle, but recent studies have shown that by thermally degrading polyethylene and polypropylene at temperatures less than 400 °C, the degradation products can be condensed into waxes without the occurrence of pyrolysis reactions or the complete degradation of small molecules. These waxes are predicted to have twice the economic value of virgin plastics and does not require expensive catalysts, presenting an opportunity for this process to be adopted commercially^{85,86}. Sulfuric acid treatment of these thermolysis products can be used to convert the waxes into alkyl hydrogensulfates, which can be used as detergents or emulsifiers⁸⁷. Plastic waste valorization is one crucial method for tackling the climate and plastic pollution crises, but it must be used in conjunction with a move away from petroleum-based materials in order for these crises to be mitigated.

Biomatter use in sustainable polymers

Algae is a feedstock that is abundant and possesses interesting macromolecules that can be used for biomaterial production. The biodiversity among different algae species presents the opportunity for unique materials based on their constituent molecules. For example, a wide variety of polysaccharides can be extracted from various species, such as cellulose, ulvan, alginate, carrageenan, agar, and fucoidan⁸⁸⁻⁹⁰. These biopolymers can be used in biocomposites as either fillers or matrices, where the properties of the material depend on the type of biopolymer used, the extraction methods used, and other components used in the mixture, as well as the processing conditions used to produce the final product.

Algal bioplastics are typically mixed with plasticizers such as glycerol and sorbitol to enable mechanical processability^{1,91-95}. While synthetic polymers have glass transition temperatures that facilitate temperature-based processing, most common biomolecules do not, meaning that traditional manufacturing techniques cannot be used without the aid of either a plasticizer or an added polymer. Polyethylene glycol (PEG) is also commonly used as a plasticizer in algal bioplastics, where PEG molecules diffuse between the polysaccharide chains and allow for chain sliding when subjected to processing conditions^{96,97}. Typical thermoplastic production methods such as compression molding and extrusion are used to

make these algal bioplastics, making them suitable for modern industrial production methods. In addition, short-range hydrogen bonding interactions occur between the hydroxyl groups on both the plasticizer and the polysaccharides, resulting in a cohesive and homogeneous matrix. Using whole biomass also avoids extraction or modification processes that can harm the environment via waste mishandling or energy intensive processes. Life Cycle Analysis (LCA) has been used to show that whole sugar kelp bioplastics have reduced impact on the environment across a range of impact factors compared to a bioplastic like PLA⁶⁴. In an effort to circumvent extraction processes, biobased and compostable materials produced from *whole* plant, bacterial, fungal, or algal biomass, without extracting components, have been reported^{1,98–106}. Whole plant materials have remarkable ranges of stiffness, strength, and toughness due to hierarchically organized structures at the nano-, micro-, and meso-scales^{100,107}. Additionally, However, purely bio-based materials can be considered unreliable or prone to wide variations in properties, so hierarchical arrangements are often destroyed via mechanical and chemical processing and the addition of polymers.

Among the studied biomass sources, algal species can be considered as a potentially disruptive material platform as they grow rapidly in a wide variety of natural aquatic environments as well as in cultures^{99,108}. This versatility may allow for algae to be grown in close proximity to facilities where they are transformed into bioplastic materials, reducing transportation emissions and costs. In addition, photosynthetic algae act as a carbon sink, thereby reducing the quantity of global atmospheric carbon dioxide.

Using the entire algal organism allows for the use of a variety of building blocks, with polysaccharides acting as structural fibers and proteins and lipids acting as reinforcements, binders, and plasticizers. Whole algae cells have been used in composites with commodity polymers as a way to reduce the environmental impact of the resulting composite. The addition of algae affects the strength and stiffness of the composites. Sapalidis, et al. showed that incorporating the algae *Zostera* as a filler in polyvinyl alcohol (PVA) led to significant increases in stiffness, improving by 1800% at a 30% filler concentration compared to the base polymer matrix¹⁰⁹. Hassan et al. showed that the incorporation of *Ulva* sp. in polypropylene (PP) improved stiffness in both tension and bending, increasing stiffness by

80% and strength by 70% at a concentration of 30 wt.% filler in matrix¹¹⁰. The addition of algae generally seems to make the composite much more brittle than the base polymer would be on its own. This embrittlement combined with stiffening may be a result of imperfect bonding between algae and polymer, leading to structural defects at the interface of the two materials. The algae particles may also decrease polymer chain mobility^{111,112}. In an effort to make fully biodegradable biofilms, Jantasrirad, et al. mixed algae into pre-gelatinized starch and analyzed the effects of sonication on the tensile strength of the films¹¹³. Using the highly abundant *Sargassum plagiophyllum*, the authors showed that ball milling the algae broke up the cell walls, causing flat, smooth particles to break into rough, angular, and smaller particles, while partially defibrillating the cellulose within the algae. The addition of 10 wt.% algae improved tensile strength the most (from 0.36 MPa to 0.67 MPa), although increasing the concentration up to 20 wt.% still produced a stronger film than pure starch. Sonicating the algae before incorporation with the starch lead to significant improvements, with increased sonication time leading to increased tensile strengths, up to 2.22 MPa for two minutes of sonication. Elongation-to-break decreased as algae concentration increased, and sonication did not significantly change that trend. Additionally, the addition of algae improved the films' abilities to an accelerated weathering test, with the biggest improvement coming from a 10 wt.% concentration of algae.

One example of algae being used as the matrix rather than the filler comes from Rizal, et al., who incorporated cinnamon nanoparticles in red seaweed (*Kappaphycus alvarezii*) films¹¹⁴. A ball mill was used to break up cinnamon powder into cinnamon nanoparticles. Combining these nanoparticles with seaweed powder and glycerol showed improvements in hydrophobicity, tensile strength and stiffness, and microbial activity inhibition. At 7 wt.% cinnamon, the water contact angle increased substantially from 51 degrees to 89 degrees, showing that the material was still hydrophobic, although markedly less so. The increase in cinnamon nanoparticle content also reduced the surface roughness up to 7 wt.% cinnamon, potentially explaining the increased water contact angle measurements. Cinnamon concentration increases did not show a clear trend of tensile strength increases, although the strongest films were achieved at 5 wt.% cinnamon. In general, the elongation-to-break

decreased with an increase in the amount of cinnamon, but at 1 wt.%, the elongation-to-break was 55%, compared to 28% without any nanoparticles. Stiffness increased with increased cinnamon concentration, plateauing at about 175 MPa at the 5 wt.% concentration. Finally, high amounts (5 wt.% and 7 wt.%) of cinnamon showed the ability to inhibit growth of *Escherichia coli* (E. coli) and Salmonella, while not having any effect on *Staphylococcus aureus* (S. aureus).

Prior work from Zeller et al.¹⁰⁵ focused on using unmodified *Arthrospira platensis* (spirulina) and *Chlorella vulgaris* (chlorella) cells to form bioplastics by subjecting them to heated compression molding. That work demonstrated thermoformability and the produced bioplastics showed tensile strengths of 5.7 and 3.0 MPa, respectively. Glycerol was also used as a plasticizer, which helped to improve the processability and improved the elongation-to-break (from 3.4% to 5.2%), while decreasing the strength and stiffness (1.6 MPa and 53 MPa, respectively) at 20 wt.% glycerol concentration.

Fredricks et al.⁹⁹ demonstrated that unmodified spirulina cells can also be used in additive manufacturing to create inks for direct ink writing. Spirulina cells suspended in water formed a slurry that could be printed and dried, where the method of drying had significant effects on the micromorphologies of the final product and mechanical properties. For example, oven drying resulted in what seemed to be gelatinization of the biomass without rupturing cells, creating a continuous matrix embedded with whole spirulina cells. Freeze-drying, meanwhile, led to the formation of a porous structure, making the material more like a foam rather than a bulk material.

The limited understanding of mechanisms to precisely transform algal biomass to bioplastics, as well as the poor mechanical properties that have been presented in prior literature, hinder the application of this material class, especially as a replacement for high-volume commodity plastics. This thesis aims to improve the fundamental understanding of algal feedstocks as a material and how algal bioplastics can be made to be competitive with commodity polymers while retaining the important characteristics of sustainability and biodegradability.

As mentioned before, cellulose is another naturally-occurring biopolymer that may be

used to create bioplastics. Cellulose is the most abundant natural polymer in the world, found in the cell walls of algae and plants, and can even be synthesized by certain strains of bacteria^{115,116}. Compared to plant-derived cellulose, bacterial cellulose (BC) offers several distinct advantages, such as a significantly higher aspect ratio of the fibers, and higher crystallinity, that contributes to it having a higher strength and stiffness^{116,117}. Moreover, BC can be produced at commercially viable volumes with high purity, reducing the need for energy-intensive extraction methods such as Kraft pulping, which is used for wood-derived cellulose¹¹⁷.

Cellulose is especially interesting as a nanomaterial due to its low cost compared to metal or petroleum-based nanomaterials. The dense packing of hydrogen bonds, longer-range Van Der Waals interactions, and fibrillar entanglements give cellulose remarkable strength (6-7 GPa) and Young's modulus (120-140 GPa)^{1,118}. Facile chemical modification and processing of cellulose has led to the creation of films with impressive mechanical properties. For example, Reyes et al. used ionic liquids to create cellulosic films with strengths approaching 300 MPa and Young's moduli over 7 GPa¹¹⁹. Yang and Berglund similarly made transparent cellulosic films using a simple degumming and alkali treatment process with an acetate buffer solution and sodium hydroxide. Treated cellulose fibers were hot-pressed into a fiber-aligned film with strengths over 500 MPa when tested in the direction of fiber alignment¹²⁰. Cellulose fibers have also been used to make cellular materials for biomedical applications and liquid absorption/filtering applications^{121,122}. These foams are often made by first making cellulosic hydrogels, then freeze-drying them. Ali and Gibson demonstrated that cellulose cryogels follow similar mechanical scaling laws to other open-cell foams and propose that a better understanding of controlling the pore morphology of cellulosic foams can lead to greatly improved mechanical properties for a variety of applications¹²³.

Fungi provide another source of polysaccharides that can be processed into useful materials. Fungi produce a fibrous network called mycelium, which is made of filamentous cells called hyphae, to colonize and consume substrates. In the process of consuming the substrate, mycelia will grow to fit the shape of the substrate over time, allowing for

a straightforward way of molding or shaping the mycelium mat. Hyphal cell walls are principally composed of chitin, which is a homopolymer comprised of $\beta(1\rightarrow4)$ -linked, N-acetylglucosamine units. These are very similar to cellulose, except for the presence of an acetamine group in place of a hydroxyl group at the C2 position¹⁰⁶. It has been shown that chitin nanocrystals can have remarkable strength and stiffness that varies based on the crystalline polymorph. The axial elastic modulus of α -chitin typically varies between 41 and 60 GPa, while β -chitin can have moduli as high as 150 GPa^{124–126}. The growth mechanism of mycelium and these attractive mechanical properties have led to the extensive exploration of mycelium-based materials.

When mycelium grows through a substrate, it consumes part of the substrate while embedding fibers into the substrate particles, creating a mycelium/substrate composite. This has been used to make foams due to the naturally porous nature of the substrate and the mycelial network. While rapeseed straw and sugarcane bagasse are common substrate materials, it has been shown that sawdust and sawdust/sugarcane composite substrates can create foams with higher density and strength, of 0.3 g/cm³ and 6.7-7.5 MPa, respectively^{127–129}. Varying the form of the feedstock also has an effect on the final material properties. For example, compressing a substrate prior to inoculation can improve the compressive stiffness (1.1 to 1.4 MPa) compared to using loose fibers (0.1 to 0.6 MPa)¹³⁰. Haneef et al. showed that changing the culture medium, for example by adding potato dextrose broth to a pure cellulose substrate, affected the final chemical and mechanical properties of mycelium films. This addition caused changes in the lipid profile of the hyphal cell walls, leading to a reduction in Young’s modulus (from 12 MPa to 4 MPa) but an increase in elongation to break (from 14% to 33%). Appels et al. modified the growth conditions of genetically-modified mycelium by using excess light and CO₂, which greatly increased the density and therefore the mechanical properties of the mycelial foams. The elastic modulus increased from 0.91 GPa to 2.73 GPa and the strength increased from 9.5 MPa to 40.4 MPa, apparently due to the higher molecular weight of the mycelial biopolymers^{1, 131}.

Proteins are a unique class of biopolymers that have long been used for bioplastic

production, although modern understanding and processing techniques have opened up new possibilities. Silk from silkworm cocoons is prepared by chopping up the fibers and removing the sericin layer by boiling them in a sodium carbonate solution. Silk fibroin can then be dissolved in a lithium bromide solution for further processing^{132,133}. Xie, M., Lian, L., and Zhang, Z. et al. demonstrated the use of silk sericin or fibroin, decellularized extracellular matrix, gelatin, and a photoinitiator for 3D printing of bioplastics that can have tunable properties depending on which component of silk is used, demonstrating use of these materials as cell scaffolds¹³⁴. Plastics have also been made from silk using thermoforming techniques. Guo et al. hot-pressed regenerated silk powder to create hard bioplastics with up to 2.5% elongation to break and flexural strengths over 150 MPa. This material became soft at temperatures above 140 °C, allowing it to be thermoformed and nanoimprinted¹³⁵. Further work on this system from Wang et al. showed that by using a water mist to plasticize the regenerated silk fibroin powder, plastics could be thermoformed at only 60 °C, creating transparent plastics that can be processed at temperatures safe for microorganisms that can turn these materials into living materials¹³³.

Bovine Serum Albumin, a commonly used protein for laboratory research, has been used in several bioplastic studies. Sanchez-Rexach et al. used Bovine Serum Albumin (BSA) proteins with polyethylene glycol diacrylate (PEG-DA), and a Ru(bpy)₃Cl photoinitiator to print bioplastics via stereolithography. Due to the plasticity afforded by the unfolding of the globular proteins, these printed plastics plastically deformed due to compression or extension, but recovered their shape upon heating or soaking in water¹³⁶. Later explorations of this material system showed that treating with tannic acid and heat could improve the properties of the printed parts. By curing at 120 °C and treating in tannic acid, the swelling ratio in water reduced from around 1.5 to almost 0. The tannic acid seems to increase the hydrogen bonding in the matrix, increasing strength by up to 27-fold¹³⁷. Fadaie et al. denatured BSA using HCl and heat, and added glutaraldehyde to crosslink the resulting solution. The hydrogels were then freeze-dried to make aerogels that were highly flexible, and able to maintain a small hysteresis loop over 10 cycles of compression to 70% strain. These foams displayed excellent dye adsorption properties,

indicating potential usefulness in water purification¹³⁸.

Proteins can work well in composites due to their diverse functionality from various amino acids and a complex structural organization. De France et al. added lysozyme to cellulose nanocrystal films and studied the effects of sonication on lysozyme structure. Lysozyme conforms in both α -helix and β -sheet arrangements, but this secondary structure was not affected by the sonication. Still, the presence of lysozyme increased toughness of CNC films by 84% at a 10 wt.% concentration of protein in CNC. Protein conformation and organization are heavily impacted by salts, which screen electrostatic charges and can physically crosslink proteins, and metallic nanoparticles, which can create protein-metal complexes. Nyström et al. combined β -lactoglobulin amyloid fibrils with gold nanoparticles using a sol-gel approach to create a hydrogel, that was then dried with supercritical CO₂ to form aerogels. The amyloid/gold dispersions remain stable for weeks due to electrostatic repulsion, but adding salt allows for the screening of these charges, leading to a sol-gel transition that produces free-standing gels. Upon drying, the resultant foams have pores on the range of 2-130 nm in diameter and densities of 1.7 to 29.6 mg/cm³, without gold. The distribution of gold throughout the foams allows the authors to make a pressure sensor, where upon sufficient pressure the gold crystals will percolate and increase the conductance of the material, up to a conductance threshold¹³⁹. Similarly, Shen et al. used amyloid fibrils and CaCO₃ nanoparticles to create hydrogels with self-healing properties and pH responsiveness. The nanoparticles provide electrostatic charge shielding to neutralize the amyloid dispersion as well as crosslinking through covalent bonds between carboxylic groups on the protein and the CaCO₃ around the nanoparticles. Rheological tests showed that the storage modulus of the gels with nanoparticles was twice as high as the control gels. It was found that changing the amount of nanoparticles acting as crosslinkers increased the density of the hydrogels. Wetting the gels and pressing them together caused cohesion of the two gels even under vigorous stirring, and oscillatory rheology confirmed that after a shear deformation of 1000%, wetting the sample with pH 2 water caused the eventual recovery of structure.

With improvements in bioengineering, synthetic biology has become a new frontier of

material development, using a bottom-up approach to grow new materials. Kim et al. designed proteins by combining zipper-forming A β amyloid proteins, MaSp1 silk proteins from dragline spider silk, and mussel foot protein-5 (Mfp5) and used genetically engineered *E. coli* to grow those proteins. This approach allows for the formation of proteins that are not available naturally and therefore can have unique properties baked into their design. These 8xKLV-Mfp5 proteins formed hydrogels in 5-150 mM sodium phosphate solutions and could be cast into different shapes for testing. Wide-angle X-ray diffraction (WAXD) showed that the Mfp5 had a negligible impact on the crystallinity of the protein structures, which were made up of 40 β -strand crystallites made up of 8 β -strand subunits. Approximately 72% to 75% of the amyloid sequences were folded into β -crystals, which is much higher than hydrogels made from silk only. The presence of the disordered Mfp5 protein domains provided extensibility compared to hydrogels made only with the highly ordered 8xKLV proteins, allowing for 100% strain in tension. Additional functionality is provided by the oxidation of tyrosine residues into dihydroxyphenylalanine (DOPA) residues present in the Mfp5 proteins, giving strong underwater adhesion properties to various substrates such as glass, plastics, and aluminum. The adhesion with glass and aluminum were strongest due to catechol-silica bidentate hydrogen bonding interactions and catechol-metal coordination¹⁴⁰. Similarly, proteins with half of an Mfp5 protein on one end of an amyloid-silk hybrid protein and the other half of an Mfp5 protein on the other end were synthesized using *E. coli*. These proteins were extruded into a bath of hexafluoroisopropanol (HFIP) to create fibers with remarkable tensile strengths of 481 MPa and toughness of 179 MJ/m³. The self-interaction of mussel foot protein fragments, via cation- π and π - π bonds, and the interactions of positively-charged residues and tyrosine, promote alignment of β -sheets in the shear direction during extrusion, strengthening and toughening the amyloid-silk fibers. This has been shown to work for other proteinaceous fibers as well, such as recombinant spider-silk and titin¹⁴¹.

Engineered living materials (ELMs) are a quickly growing research topic that is building from all of the knowledge on synthetic and biological polymer outlined so far. In ELMs, polymers serve as a substrate for microbial activity that can then confer various

functions to the material. ELMs have been studied for a variety of purposes including carbon capture, drug delivery, and structural materials. Wild-type cyanobacteria, which fix CO₂ through photosynthesis, and *E. coli* have been seeded into porous, moisture-wicking ceramics to capture CO₂ 2.9x faster than liquid cultures due to the high surface area of the porous ceramic scaffold¹⁴². Similarly, a 3D-printed scaffold of P407 hydrogel embedded with *Synechococcus* PCC 7002 both captured CO₂ and precipitated CaCO₃, hardening the structure. Over 400 days, the hydrogel captured 26 mg of CO₂ per gram of photosynthetic living material via mineral formation alone¹⁴³. A hybrid ELM consisting of *Synechococcus elongatus* and *Lactococcus lactis* encapsulated in a PEG-DA matrix cured alongside chitosan using UV light was researched for use as a wound dressing. *L. lactis* was genetically modified with enhanced expression of chemokine 12, reducing the inflammatory response and promoting macrophage recruitment to the wound site, promoting wound healing. Tests on rat skin wounds showed complete healing within 14 days, highlighting the potential for ELMs as therapeutic agents¹⁴⁴. A new category of ELMs, termed stiff living materials (SLMs), include materials created by drying out the bacterial and their biofilms. By casting a highly concentrated wet mass of cells, such as *E. coli*, *Lactobacillus rhamnosus*, and *Saccharomyces cerevisiae*, a film made up of whole cells can be made with some of the cells still alive. Manjula-Basavanna et al. found that *E. coli* and *S. cerevisiae* films had approximately 35% of the cell mass still living after casting, while *L. rhamnosus* had no living cells remaining. Nanoindentation was used to determine a range of Young's moduli from 5 to 42 GPa for all SLMs in this study, including a film made of ethanolysed *E. coli* cells. The stiffness of these materials indicate that cellular components can self-assemble to form stiff materials, even if the assemblage is heterogeneous. For the SLMs with living cells in them, pieces of film could be broken off and re-cultured, allowing for the production of many "generations" of SLM with each successive generation having similar properties to the original (for up to 3 generations in this study)¹⁰¹.

Similarly, Duraj-Thatte et al. created biofilms from genetically engineered *E. coli*, which synthesize CsgA-based curli fibers in a hydrogel network. The hydrogel is then mixed with sodium dodecyl sulfate (SDS) to induce gelation, after which it is cast in a

mold and dried in ambient conditions. With a tensile strength of 18 MPa and Young’s modulus of 1 GPa, these films are categorized as SLMs. The presence of living *E. coli* confers self-healing properties, which is one of the big draws of living materials. Scratches and full-thickness cuts made into the films were completely repaired by spraying water onto the damaged areas. However, the Young’s modulus and strength decreased by 40% and 33%, respectively, after the healing¹⁰².

Green chemistry strategies for chemical modification of cellulose

To improve the strength of cellulosic materials, chemical treatments have been used as a way to create nanofibers and nanocrystals, or to simply defibrillate the cellulose fibers^{145,146}. Deep eutectic solvents (DES) offer a promising, environmentally-friendly alternative to conventional acid hydrolysis treatments of cellulose, avoiding the use of harsh acids^{147–149}. Prior literature has suggested that using various DES treatments on cellulose can nanofibrillate BC and increase the absolute zeta potential, allowing for more colloidally stable suspensions. Some literature has shown that DES can also be used to add functional groups to the cellulose^{147,150,151}.

For example, Henschen et al. demonstrated that esterification and acid hydrolysis of cellulose with oxalic acid dihydrate resulted in improved mechanical properties of cellulose films due to the nano-fibrillation of cellulose fibers¹⁵². Similarly, Nguyen et al. developed an adsorbent foam for the removal of wastewater dyes based on the crosslinking of BC using citric acid¹⁵³. DES has also been used with cellulose from both plants and bacteria as a more sustainable solvent for producing a variety of foams and aerogels^{154–158}. Farooq et al. extracted microfibers from *Firmiana simplex* bark and used a DES to make aerogels that provided more than 90% purification efficiencies for air pollutants¹⁵⁴. Similarly, Laitinen et al. used DES to defibrillate kraft pulp before a silylation process that allowed for the creation of hydrophobic aerogels with the ability to absorb marine diesel from water at capacities of up to 142.9 grams of diesel per gram of aerogel¹⁵⁶. Long et al. also used DES and carbon nanofibers (CNFs), along with carbonation, to produce an aerogel filter with a volumetric absorption capability for organic pollutants and oils from water of 74–95%¹⁵⁷. For a sensing application, Panikar et al. used DES to fabricate gold nanoparticles

on a cellulose nanocrystal (CNC) scaffold. This composite was able to detect pesticides in rice and tea extracts and Methylene Blue in fish muscle extract, although sensitivity was dependent on nanoparticle morphology¹⁵⁵. Recently, PVA, palm wax, and cellulose foams were created using DES by Li et al., with the DES treatment allowing for a high cellulose yield from the cotton fibers that were the feedstock for the study¹⁵⁸. They found that the combination of these materials resulted in a hydrophobic foam that may be an option for packaging materials in the future. Based on these studies, DES is typically used to treat cellulosic materials before other processes, and the resultant aerogels have largely been studied for filtering applications. Only one of these studies¹⁵⁸ focused on properties relevant to packaging applications.

Another approach to alter the properties of cellulosic materials is to combine it with similarly sustainable biopolymers to form networks that can then be processed into different forms from hydrogels to bulk solids or foams. Pectin, for instance, is a critical component in the cell walls of terrestrial plants, acting as a compliant matrix for the stiff load-bearing cellulose fibers. Industrially, it is often used in food as a gelling agent, giving structure and recoverability to food products. It can also be used as a food stabilizer, helping to keep fruit components, such as pulp fibers, from separating out in a mix. The ability for pectin to improve recoverability and aid colloidal stability, as well as its natural presence in plant cell walls, makes it a promising candidate as an additive to cellulosic materials. Cellulose and pectin is therefore a material system that has attracted significant interest, and has been well-studied in both colloidal and solid states. Examples include cellulose/pectin hydrogels and colloids^{159,160}, films¹⁶¹, and foams¹⁶². BC specifically has also been studied with pectin, typically in the form of pellicles or films with the pectin added in the culture medium in an effort to better understand natural polymerization reactions or biopolymer functions in plant cells^{163,164} or to create composite films with compelling mechanical properties¹⁶⁵.

Most materials that have been discussed so far that are made from whole biomatter are really only made from a small number of biomolecules, such as pure silk or cellulose/pectin composites. However, there remains a gap in knowledge for how processing methods and conditions affect the resultant materials made from complex combinations of biomass, such

as whole plant cells or blades of algae. The following thesis is a step towards closing that gap for bulk bioplastics and cellular biofoams.

1.3. Thesis Overview

To produce sustainable and biodegradable bioplastics, we will be using the abundant and complex set of biopolymers found in algae. As stated earlier, the abundance of algae and speed of growth makes it a promising candidate for making bioplastics^{99,108}. There is also a wide variety of algae and therefore a large amount of molecular variation to work with to produce interesting materials. In my first aim, I will use spirulina, a single-celled cyanobacteria colloquially referred to as an algae, as a material for bulk bioplastics. In Aim 2, I will use a model binary carbohydrate system comprising of bacterial cellulose and pectin in order to study and utilize interactions between those different biopolymers at the colloidal state to control structure and properties of resulting foams. Finally, in Aim 3, I will use algae to make foams for acoustic absorption, leveraging material and acoustic data to develop models for the production of acoustic absorption metamaterials. Throughout this thesis, the difficulties of processing and characterizing materials made from whole biomass will be a consistent theme that deserves further attention in the future.

1.3.1 Aim 1: Processing-structure-property relationships in cohesive bioplastics from unprocessed biomatter

Here we aim to study the processing-structure-properties relationships of bioplastics obtained from “whole” biomatter - specifically spirulina cells. First we aim to understand the effects of thermomechanical processing conditions (temperature, pressure, time) on the mechanical properties and microstructure of the obtained bioplastics, without the use of any binders or additives. We hypothesize that the processing conditions will enable different morphologies and bonding motifs within the biomatter which sequentially will result in different macroscopic mechanical properties.

Next, we will modify the mechanical performance of the bioplastics using additives. We intend to study additives which will be safe for degradation in the environment, so that the

ultimate end-of-life options are maintained. We hypothesize that fillers such as nanoclays and cellulosic or mycelium fibers, can act as naturally-derived nanoreinforcing agents in the bioplastics, improving the strength and toughness. On the other hand, natural polyols (low molecular weight sugars) such as sorbitol and glycerol, are expected to improve ductility and toughness.

Finally, we will study the biodegradation mechanisms of our algal bioplastics. Algae readily absorbs water and can be consumed by microorganisms, making it fully biodegradable in soil. Once the algae are converted to bioplastics, however, there may be chemical changes that affect that degradation process. Therefore, the final stage of this thesis will be to understand and quantify the biodegradation kinetics and mechanisms in the bioplastics and how changes in processing and additives affect those. We will consider several established kinetics models to describe the degradation, such as the n^{th} order $[(1 - \alpha)^n]$, contracting volume $[3(1 - \alpha)^{2/3}]$, or n^{th} order with autocatalysis $[(1 - \alpha)^n(k_1 + k_2\alpha^m)]$ models. We anticipate that degradation will occur mainly through hydrolysis and microbial action, which is likely to be affected by swelling and hydrolysis of the materials while in damp soil. Besides biodegradation, recycling and reuse methods for algal bioplastics will be explored as well.

1.3.2 Aim 2: Assessing the impacts of colloidal-state properties on the solid state of mixed-solubility carbohydrate binary networks

For our second aim, we will investigate the colloidal and solid-state interactions between two carbohydrates that represent water soluble and insoluble biopolymers in algal biomatter. This will provide a more controlled system to understand how colloidal properties connect to solid properties, and this simple system will help to gain some insight into how different molecules might behave in algal systems. First, we will investigate the effects of treating BC dispersions with a citric acid and choline chloride DES, and how that treatment alters electrostatic, rheological, colloidal, morphological, and mechanical properties of the BC dispersions and the foams made from lyophilizing them. We will then add pectin

to the BC dispersions and study how the pectin changes those properties. We then will highlight how the changes to the BC-based materials and their colloidal properties affect the properties of the resulting freeze-dried foams. Finally, we will compare the BC/pectin composites with commercial foams such as EPS, PE, and PU, which are commonly used in packaging applications. We will investigate if biomatter-based foams can be competitive with petroleum-derived foams for such applications, providing a potential path toward sustainability for the packaging sector. The understanding of the behavior and formation of this network of soluble and non-soluble carbohydrates in water informs my next study.

1.3.3 Aim 3: Understanding effects of biopolymer interactions on the structure of whole algal biomatter foams to enable applications in acoustic absorption devices

Here, we will investigate the use of whole biomatter to form biodegradable and self-extinguishing foams from algae with a complex array of soluble and non-soluble biopolymers, including the carbohydrates cellulose and ulvans, respectively. We will lyophilize different species of algae to create foams with different sound absorption properties as measured by an impedance tube. Compositional differences between three different species of algae, *U. ohnoi*, *U. lactuca*, and an unknown species *U. sp*, will be explored to understand how changes in composition affect material properties at different steps of the fabrication process. Rheology of the pre-lyophilized algae will be used to gain insight into how the processing of the algae slurries affects the behavior of the resultant algal gels, as well as how the shear behavior of those gels relate to the mechanical and morphological properties of the freeze-dried foams. Compression will be used to understand the static and dynamic mechanical properties of the lyophilized foams. Scanning electron microscopy (SEM) and micro computed tomography (micro-CT) will be used to determine the morphological differences between each material caused by differences in processing, density, and composition of each species. The acoustic absorption properties will be measured by a standardized impedance tube setup, and this experimental data will be fitted to a semi-phenomenological model that will allow for the extraction of visco-thermal-inertial

parameters within the cellular materials. Finally, the biodegradation in seawater and flammability of the algal foams will be assessed, along with a discussion of the limitations of current methods for analyzing bioplastic biodegradation.

2. Structure and Properties of Spirulina Bioplastics

This chapter is based on the following published work:

Hareesh Iyer, Paul Grandgeorge, Andrew M. Jimenez, Ian R. Campbell, Mallory Parker, Michael Holden, Mathangi Venkatesh, Marissa Nelsen, Bichlien Nguyen, Eleftheria Roumeli. Fabricating Strong and Stiff Bioplastics from Whole Spirulina Cells. *Advanced Functional Material*, 33(40):2302067. Publisher: Wiley-VCH

2.1. Materials and Methods

2.1.1 Materials

Organic spirulina was purchased from Nuts.com. Sorbitol and MMT nanoplatelets were purchased from Sigma Aldrich, USA. Bacterial cellulose was produced from a kombucha culture as reported in¹⁶⁶.

2.1.2 Sample Fabrication

An Analog Vortex Mixer from VWR was used for initial powder mixing. The premixed powders were then subjected to a compression molding process on a TMAX-SYP-600 hot press from TMAXCN, using custom-made stainless steel molds. As shown in Figure 2.1, the hot press has two platens that are heated. The temperature is set by two control boxes, but a thermocouple was used to ensure the platens were at the correct temperature.

A calibration was performed to determine what to set the temperature of the hot press to in order to achieve the desired temperature. Figure 2.2 shows the linear trend line that was used to determine hot press set temperatures, with values from Table 2.1 showing the

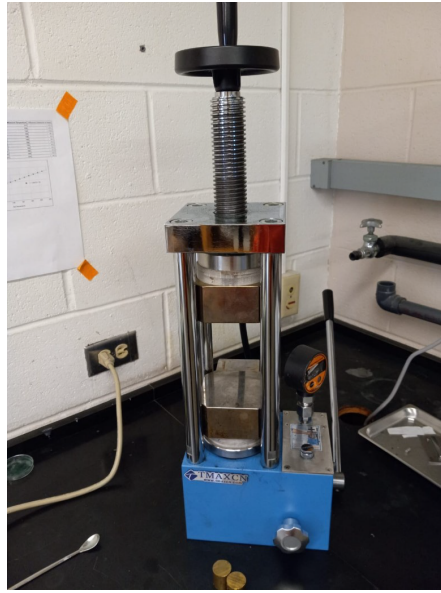


Figure 2.1: TMAX-SYP-600 hot press used to produce samples for these experiments.

set and recorded temperatures. Equation 2.1 shows the linear equation that was used for approximating the real temperature based on the temperature set on the hot press.

$$\text{Measured Temperature} = 0.979 * \text{Set Temperature} + 12.9 \quad (2.1)$$

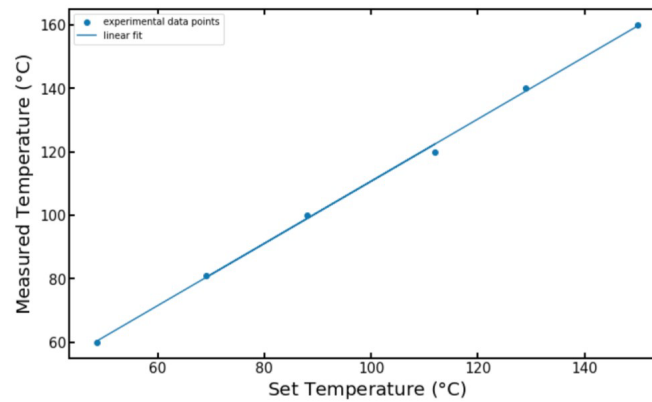


Figure 2.2: Calibration curve for the hot press temperatures.

To press the samples, a three-part mold was designed and machined. The material for the mold was stainless steel, which was chosen for increased durability over aluminum, a quality that was important to consider when looking at the number of samples that were required for this study. An exploded view of the 3D model for this mold is shown in Figure 2.3, created using Autodesk Fusion 360.

Set Temperature (°C)	Measured Temperature (°C)
48.5	60
69	81
88	100
112	120
129	140
150	160

Table 2.1: Temperatures measured from the calibration of the hot press

Because of the low thermal conductivity of stainless steel, the mold pieces were heated on a hot plate to approximately the same temperature as the hot press. This pre-heating step ensured that the powder would spend the vast majority of its time in the mold at the intended temperature. The pure spirulina specimens were fabricated by placing the short bottom piece of the mold in the slot in the middle piece of the mold. The resulting cavity was then filled with one gram of spirulina powder, making sure that the powder is evenly spread out throughout the mold. Finally, the tall top piece was inserted into the cavity, compressing the powder between the top and bottom pieces. The filled mold assembly was then placed between the hot plate platens set to the proper temperature, the platens were tightened around the mold, and the pressure reading on the hot press was set to the appropriate reading based on the required pressing force. Once the pressure was reached, a timer was started, and the pressure was held for the entire duration. The molds were allowed to cool to handling temperatures before the specimens were removed. While cooling, the mold was placed between aluminum blocks that acted as heat sinks as well as weights to ensure the specimens remained flat while cooling.

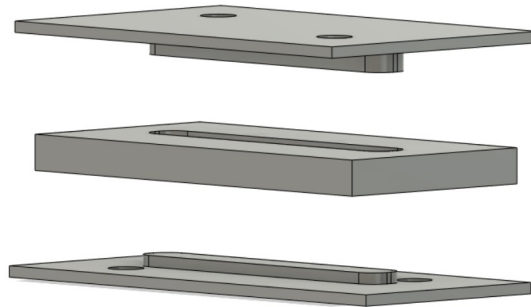


Figure 2.3: CAD model of three-point bend specimen molds.

While the hot press reads out a pressure value when pressing samples, that pressure is not transferrable to hot pressing machines of different sizes. Therefore, a desired pressing force was used, and converted to an appropriate pressure reading from the hot press. The piston diameter in the hot press was 95 mm in diameter. This diameter was used to convert the desired pressing force to a pressure reading on the hot press, as displayed in table 2.2.

Force (kN)	Pressure Reading on Hot Press (MPa)
2	0.28
7	0.99
20	2.82
35	4.94

Table 2.2: Conversion From Hot-Press Force to Pressure on the Hot Press Reading

For further reproducibility, Table 2.3 shows the pressures that were applied on the powder at each pressing force.

Force (kN)	Pressure on Powder (MPa)
2	4.3
7	14.9
20	42.6
35	74.5

Table 2.3: Conversion From Hot-Press Force to Pressure on the Powder

To examine the effects of temperature and pressure on the pure bioplastics, temperatures ranged from 60 to 160 °C by steps of 20 °C, while pressing forces of 2, 7, 20, and 35 kN were used.

For the bioplastics from dissociated spirulina, an aqueous suspension of the as received cells in a 1:10 w/w concentration was sonicated for 20 min using a Fisher Scientific Model 505 probe sonicator with an ice bath. After sonication, the dissociated biomatter suspension was freeze-dried using a Freezone lyophilizer from Labcono Corp. For the spirulina/nanoclay composites, the pre-sonicated and freeze-dried dissociated spirulina powder was mixed with the nanoclay powder at the desired concentrations prior to being hot pressed. To produce spirulina/BC composites, BC sheets with dimensions of 40 mm by 1

mm were laid along the XY plane of the mold, in between pure spirulina powder, creating a layered structure that was hot pressed at 140 °C/7 kN. The BC sheets made up 10 wt.% of the biocomposite. To prepare the spirulina/sorbitol biocomposites, the premixed powders at the desired concentrations of 1, 5, 10, and 30 wt.% sorbitol were first compounded on a Scientific Process 11 Twin-Screw Extruder from Thermo Fisher, operating at 40 RPM, with a uniform temperature profile of 90 °C, before being hot pressed at 80 °C and 7 kN, for 1 minute.

2.1.3 Characterization

Mechanical Testing

Three-point bend specimens were desiccated for 24 hours at 23 °C, before being tested on an AGS-X test frame from Shimadzu Scientific Instruments. A minimum of 9 samples were tested for each composition at a 0.5% per second strain rate and 40 mm gauge length. Compression testing was performed using an Instron 4505 universal test frame with a 5500R upgrade. A 100 kN load cell was used. A minimum of 5 samples per testing direction were tested at 0.5% per second strain rate.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was conducted on an Apreo VP from ThermoFisher Scientific, on samples previously sputter coated with 4 nm of platinum on an EM ACE600, produced by Leica Microsystems.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) was performed using a Thermo Scientific Nicolet iS10 FTIR in ATR mode. Spectra were obtained with a resolution of 2 cm⁻¹ and 128 scans. Scans were performed between 400 and 4000 cm⁻¹. Spectra were vector normalized and distributed vertically for ease of comparison. Peak identification was performed using the `scipy.signal.find_peaks` function in Python.

X-Ray Photoelectron Spectroscopy

All x-ray photoelectron spectroscopy (XPS) spectra were taken on a Kratos Axis-Ultra DLD spectrometer. This instrument has a monochromatized Al K α X-ray and a low energy electron flood gun for charge neutralization. X-ray spot size for these acquisitions was on the order of 700 x 300 μm . Pressure in the analytical chamber during spectral acquisition was less than 5×10^{-9} Torr. Pass energy for survey and detailed spectra (composition) was 80 eV. Pass energy for the high resolution spectra was 20 eV. The take-off angle (the angle between the sample normal and the input axis of the energy analyzer) was 0 degrees (0 degree take-off angle, ~ 100 Å sampling depth). CasaXPS¹⁶⁷ was used to peak fit the high resolution spectra. For the high-resolution spectra, a Shirley background was used and all binding energies were referenced to the C 1s C-C bonds at 285.0 eV.

Elemental Analysis and Protein Content Calculation

The concentration of carbon, hydrogen, and nitrogen in spirulina powder was assessed using a CHN Analyzer 2400 Model from PerkinElmer operating in combustion mode at 925 °C. The protein content of spirulina can be calculated qualitatively as the product of the nitrogen content and an indirect conversion factor. The factor used most commonly is 6.25, however, quantitative measurements of protein content indicate that the use of this factor may result in overestimation of the protein content of most algae. In fact, Angell et al. propose that 5 may be a more accurate conversion factor for seaweeds¹⁶⁸. To take into account the uncertainty of qualitative, indirect conversion from nitrogen content, we present the protein content as a range of compositions calculated using both customary and conservative conversion factors.

Thermal Analysis

Thermogravimetric analysis (TGA) was performed on a Discovery TGA 550, from TA Instruments. Samples of 13 ± 4 mg of each material were subjected to heating from room temperature to 1000 °C at a heating rate of 10 °C/min in a nitrogen gas flow of 25 $\mu\text{L}/\text{min}$. Differential Scanning Calorimetry (DSC) was done on a Discovery 2500 DSC from TA Instruments, in hermetically sealed TZero aluminum pans. Each specimen went through

two heating and cooling cycles each, at rates of 10 °C/min, with isothermal holds of 1 minute between each cycle, from -75 °C to 200 °C.

Profilometry

Optical profilometry was conducted using an Olympus OLS4100, from Olympus IMS at a 20X objective. Contact profilometry was conducted using a Bruker DektakXT, from Bruker in Billerica, MA, USA.

Biodegradation

The soil biodegradation study was performed by burying a total of 36 samples with dimensions $5 \times 5 \times 1$ mm³ of each material in gardening soil which was regularly watered to keep wet. At every time step, a set of 4 samples was recovered to measure their mass loss after cleaning them in deionized water and drying them in an oven (60 °C), for 48 hours to obtain the dry weight.

2.2. Compression Molding of Spirulina Bioplastics

Starting from raw spirulina, an abundant and commercially available microalgal species (Figure 2.4a-b), we developed a manufacturing process that enables the utilization of the entire microorganism without extraction or chemical modification processes. Upon the application of heat and pressure at optimal conditions (Figure 2.4c), the spirulina powder bonds into a rigid, thermoformable bioplastic that can be further processed and machined like a thermoplastic.

In this Chapter we will report the structure and mechanical properties of our bioplastics. By systematically varying the pressing time, temperature, and pressure (Figure 2.4d), we modulated the mechanical properties of the obtained bioplastics by controlling the micromorphology and bonding of the cell matrix. The optimized bioplastics reached strengths and moduli comparable or higher than those of commodity plastics and surpass those of TPS or previously reported algal bioplastics, while being processable with existing polymer manufacturing infrastructure (Figure 2.4e), while being backyard compostable (Figure 2.4f).

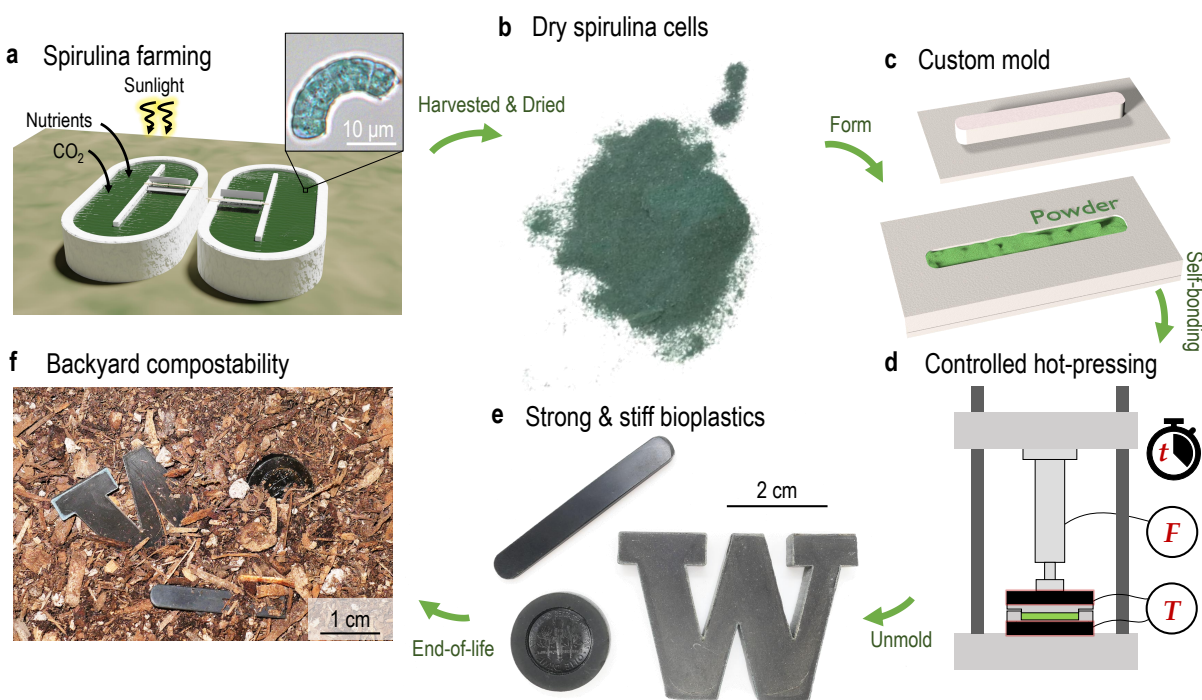


Figure 2.4: **From spirulina to biodegradable bioplastics.** **a**, Spirulina cells are cultivated at scale for commercial applications. **b**, Commercial spirulina powder used in this study. **c**, Conventional manufacturing techniques such as compression molding in a hot-press can be used to turn spirulina cells into **d** bioplastics that are **e** backyard compostable in soil.

2.2.1 Density Changes

The first step of the mechanical testing process was to measure the mass, length, width, and height of each specimen so that the density could be calculated. Based on the design of the mold, an approximation of the density had to be made. As can be seen in Figure 2.5, the mold has an obround slot shape, but the ends of the slot are not perfect semicircles. Instead, each end is two arcs of radius 3.5 mm connected by a short line of 1.0 mm. This ideal case is never achieved by the actual pressed samples, and it is difficult to measure the radius of the arcs and distance between them in a real sample. In order to complete the density calculation, an assumption that the 1 mm separation was true for all parts was used.

Density was plotted versus temperature and pressing force to examine any relationships between the three variables, as shown in Figure 2.6. In the Density vs Temperature plot on the left, it is generally observed that as temperature increases, the density of specimens

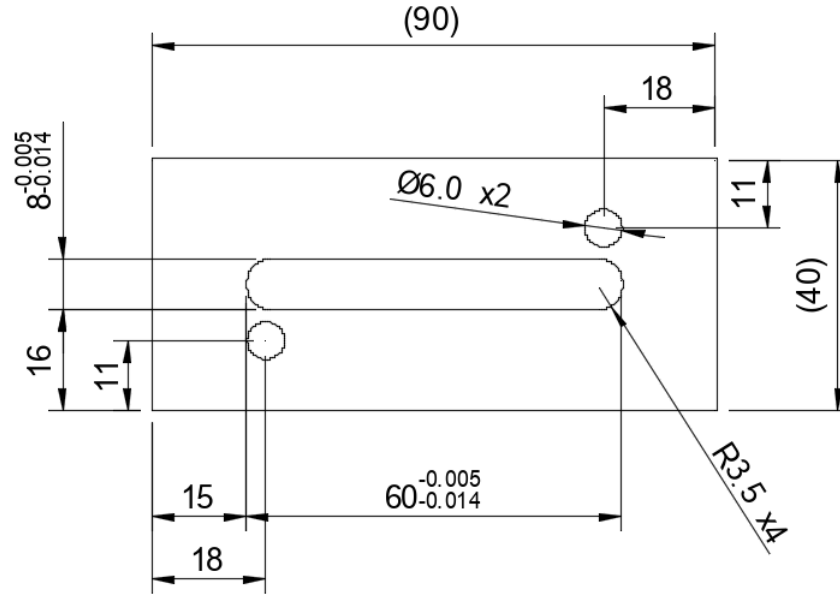


Figure 2.5: 2D schematic of the middle piece of the 3-piece mold.

approaches approximately 1.2 g/cm^3 . At lower temperatures, the scatter in densities can be explained by the lack of self-bonding in the specimens. The materials are a packed powder with loose bonding rather than a cohesive piece, so the force at which the samples are pressed has a higher impact on the density. In the Density vs Pressing Force plot (Figure 2.6), a similar trend is observed. Density generally does not change significantly as pressing force is increased except for at the lowest of temperatures, while the densities see greater change from temperature line to temperature line.

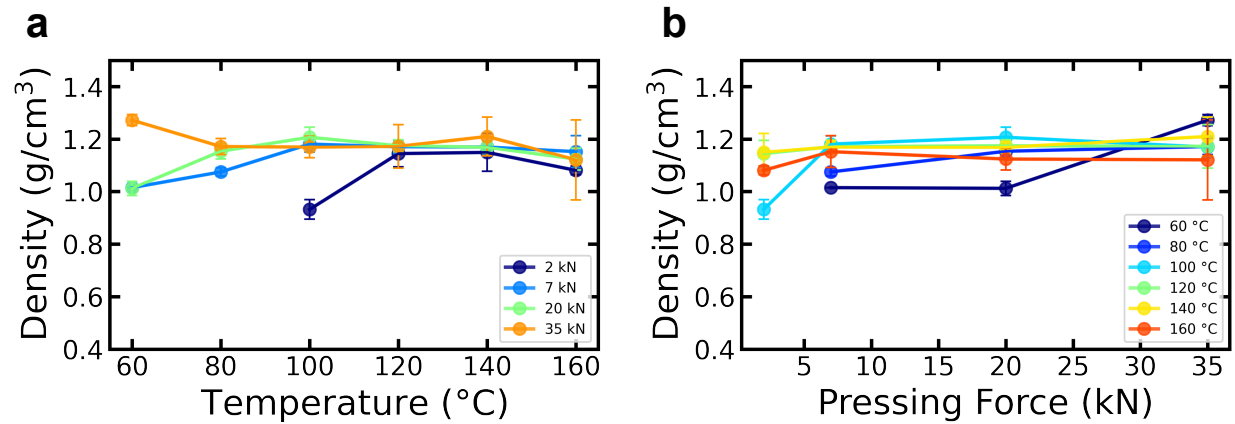


Figure 2.6: a) Density vs Temperature at different pressures. b) Density vs pressing force at different temperatures.

To investigate the effects of powder-packing on our samples, we analyzed the density vs the strength of the materials. Figure 2.7 shows a plot of error bars in both the strength and the density axes, with the center of the error bars being the average value coordinate. This plot shows the large amount of grouping around the 1.1 to 1.2 g/cm³ density values, with strengths varying significantly in that range. Meanwhile, some samples are significantly less dense, with one outlier being more dense but also less strong. All this data indicates that it is not merely powder packing that is affecting the strength of these materials, there is an actual change going on that leads to a fully self-bonded part that can be tuned higher or lower in strength and toughness based on hot pressing parameters.

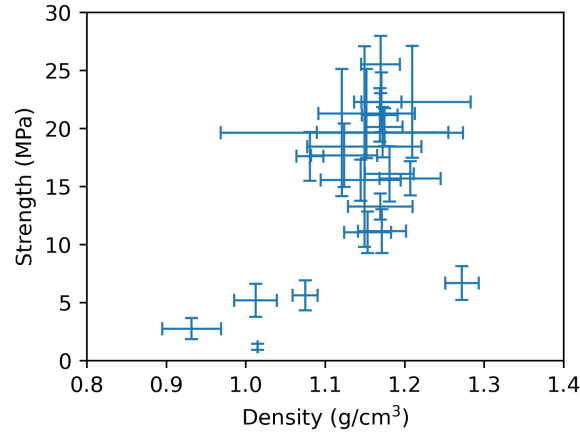


Figure 2.7: Strength vs density, with error bars showing the standard deviation in both axes.

2.2.2 Mechanical Property Optimization

To optimize the pressing conditions for the fabrication of strong, pure spirulina bioplastics, we first studied the effects of varying the processing conditions (pressing time, temperature, and force) on the resulting mechanical properties and morphology of spirulina bioplastics. First, we analyzed the effect of progressively varying the pressing time, t , between 0 and 1800 s (30 min) under fixed temperature and pressing force conditions of $T = 120$ °C and $F = 7$ kN (corresponding to an applied pressure of 14.9 MPa). We then conducted flexural tests on the resulting samples, for which representative stress-strain curves are presented in Figure 2.8a. Note that the pressing time was started once the desired pressing force was reached.

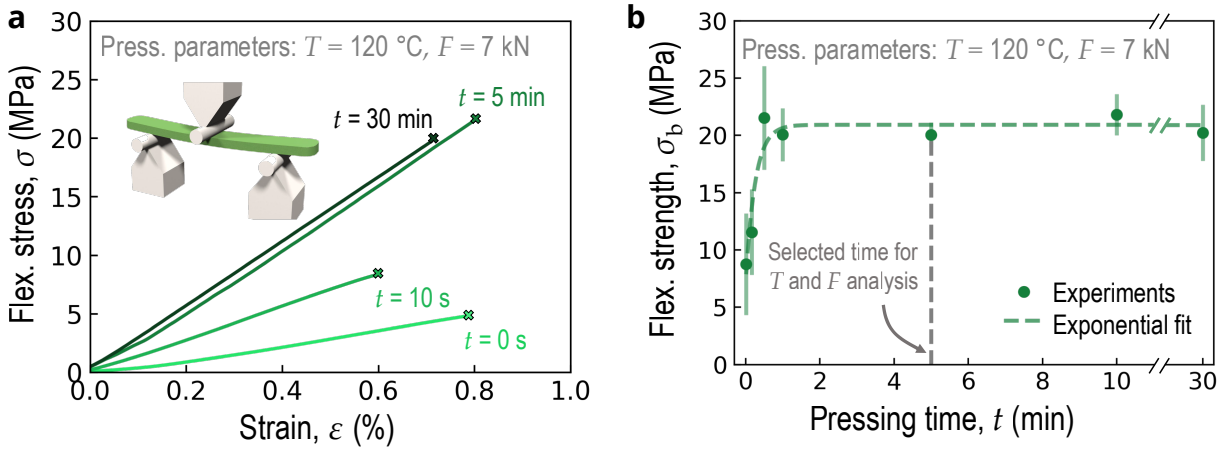


Figure 2.8: **a**, Representative stress-strain curves for spirulina at different pressing times. **b**, Flexural strength of spirulina bioplastics with varying pressing times.

The flexural strength (σ_b) is reported as a function of pressing time in Figure 2.8b, along with an exponential fit (solid line) of the form:

$$\sigma^*(t) = \sigma_0 + (\sigma_p - \sigma_0) (1 - \exp^{-t/\tau}) \quad (2.2)$$

According to this fit, the strength increases from $\sigma_{t=0} = 8.8$ MPa up to a plateau value of approximately $\sigma_p = 20.9$ MPa with a characteristic time constant $\tau = 15.4$ s. At pressing times below 60 s ($\approx 4\tau$), the strength is significantly lower than the plateau strength and the data exhibit higher standard deviations than at longer pressing times. Below this threshold, the pressing time is not sufficient to facilitate the formation of a bonded uniform spirulina matrix. This lack of self-bonding may either be caused by insufficient time for the mold and spirulina to reach the set temperature or because the necessary reactions leading to self-bonding require more thermal energy input and time to complete.

Next, we investigated the effects of pressing temperature and force on the mechanical properties of the bioplastics formed. For each value of T and F , the pressing time was fixed to the conservative value of 300 s (20τ , dotted line in Figure 2.8b), to ensure that the strength plateau could be reached and to accommodate for lower pressing temperatures and forces than the ones used in the time-variant experiment. At that fixed pressing time, we varied the temperature from 60 to 160 °C and the pressing force from 2 to 35 kN (corresponding to applied pressures in the range 4.3 to 74.5 MPa, see 2.3 for force

to pressure conversions). In Figure 2.9a, we present a heat-map of the measured flexural strength values for each of the tested temperature-pressure conditions. A region of maximal strength is observed for temperatures between 120-160 °C and a pressing force of 7 kN.

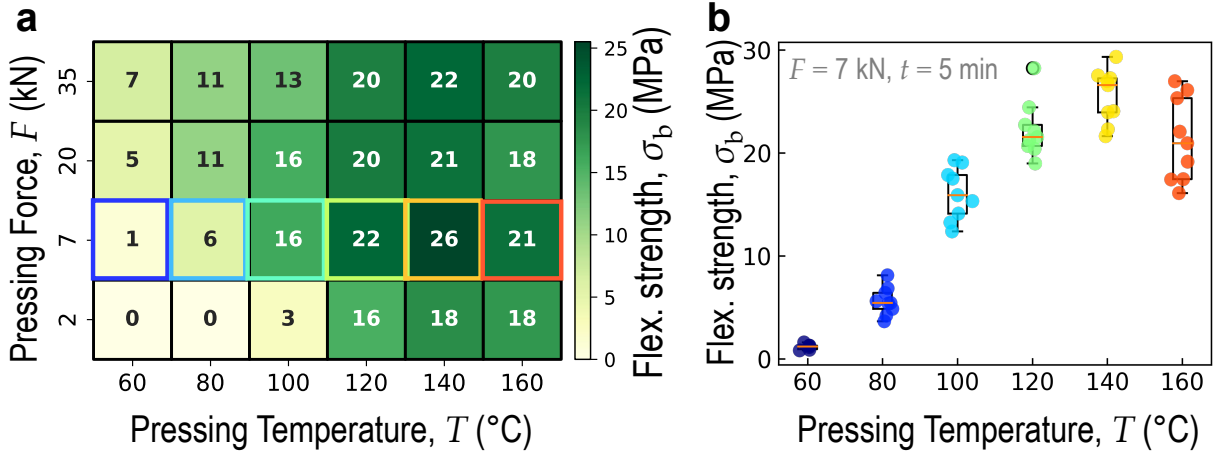


Figure 2.9: **a**, Heat map of flexural strength for spirulina bioplastics at each temperature/pressure range. **b**, Flexural strength versus pressing temperature for a fixed pressing force (7 kN, corresponding to a pressure of 14.9 MPa) and time (5 minutes).

Figure 2.9b shows the relationship between flexural strength and pressing temperature at a force of 7 kN. We observe a progressive strength increase with increasing temperature up to a maximum value at 140 °C, beyond which strength decreases. This drop in strength at high temperatures (160 °C), is seen for all pressing forces at or above 7 kN and can be attributed to the initiation of thermal degradation reactions in spirulina. Indeed, the thermal degradation profile of spirulina (presented in Figure 2.10) shows that degradation starts at around 180 °C. Figure 2.13c and d show more detail about how temperature and pressure affect the flexural strength. At the lowest pressing force of 2 kN there is a significant increase in flexural strength as the temperature increases from 100 to 120 °C (from 2.75 ± 0.90 to 15.54 ± 1.78 MPa), at which point a plateau is reached in strength. At a pressing force of 7 kN, the strength increases from 1.17 ± 0.27 MPa to 25.52 ± 2.47 MPa. The strength then drops to 21.28 ± 3.85 MPa when pressed at 160 °C, which may indicate the onset of degradation, although the overlap in error bars between those two temperatures makes it hard to determine if that is truly the case. At 20 and 35 kN pressing forces, the low temperature strengths were higher than at the lower pressing forces (5.18 ± 1.43 MPa and

6.68 $\pm$ 1.46 MPa, respectively), but they still see significant increases at 120 °C, at which point the strengths plateau at 20.10 $\pm$ 1.63 and 19.67 $\pm$ MPa, respectively. Figure 2.13 clearly shows that a pressing temperature of 140 °C consistently produces higher average flexural strengths, while 160 °C generally produces lower average strengths than at 120 and 140 °C, again most likely due to the onset of thermal degradation. The maximum strength of 25.5 MPa is reached at pressing conditions of 140 °C and 7 kN. While the degradation temperature can explain the downward trend of the data after 140 °C, it is also noticed that the 7 kN pressing condition produced higher strengths than the higher pressing force conditions. This may be explained by the observation that higher pressing conditions caused the spirulina to flow out of the mold, leading to thinner, less easily manufacturable parts. The demolding process for these specimens was difficult as the specimens ended up being rather fragile. 7 kN appears to be an acceptable compromise between having enough force to get a high strength value out of the material, but not so strong so as to disrupt the manufacturing process.

The bending modulus follows similar trends as the strength (Figure 2.13a and b). For the lowest pressing force (2 kN), a substantial increase in flexural modulus (from 0.68 $\pm$ 0.19 to 2.04 $\pm$ 0.47 GPa) was found as the temperature increased from 100 to 120 °C, which was maintained for the 140 °C and was slightly reduced (to 1.69 $\pm$ 0.23 GPa) at 160 °C. This significant increase-plateau-decrease trend was only observed to that extent at the minimum pressing force (2 kN), indicating that at this low pressure condition, a temperature of 120-140 °C is required for the samples to reach the maximum flexural modulus. At lower temperatures, the loose packing of the spirulina powder results in poorly bonded bioplastics, while at higher temperatures thermal degradation initiation may be negatively affecting the sample performance. At a higher pressing force of 7 kN, we observe the same substantial increase in modulus (from 0.35 $\pm$ 0.12 to 2.39 $\pm$ 0.29 GPa) with temperature increasing from 60 to 100 °C. Similar modulus fluctuations exist but are smaller at a higher pressing force of 20 kN. A small increase (from 1.18 $\pm$ 0.31 to 2.22 $\pm$ 0.16 GPa) is observed when temperature is increased from 60 to 80 °C and a plateau is reached above that threshold. At a maximum pressing force of 35 kN, the plateau is obtained even at

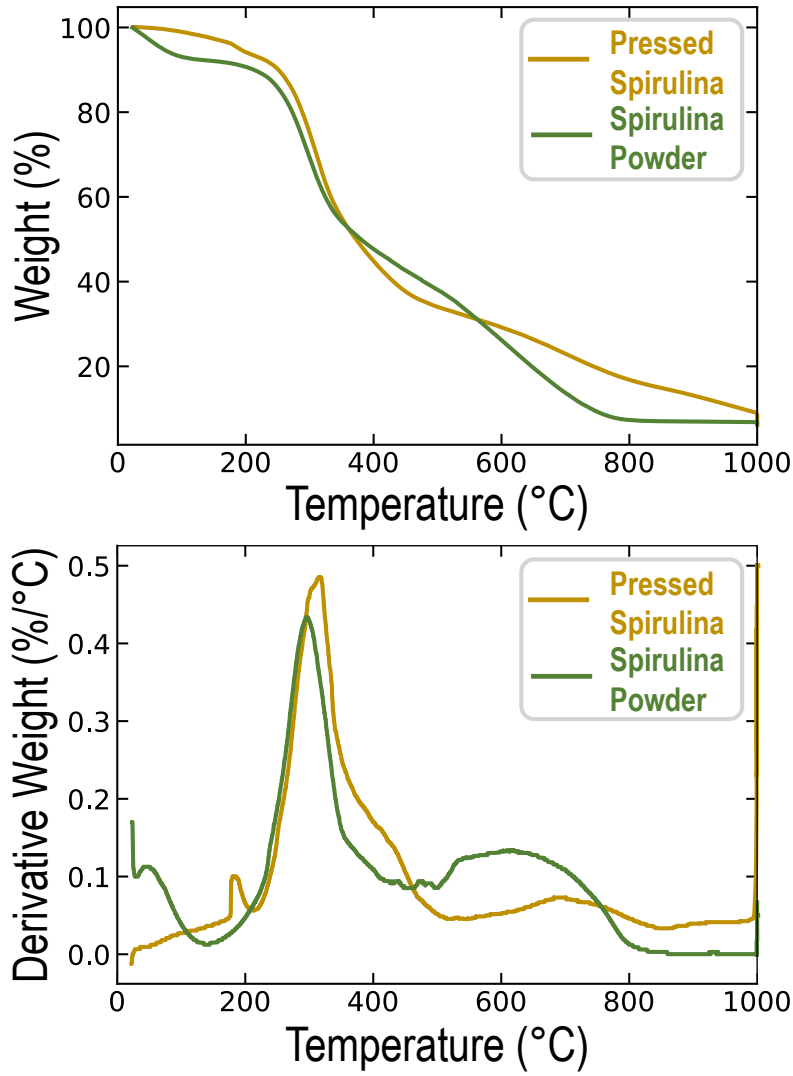


Figure 2.10: Representative curves for the thermogravimetric curves (top) and derivative of the thermogravimetric curves (bottom) for pressed and powdered spirulina.

60 °C but the larger deviations for each sample indicate that the degree of uniformity in the samples is more variable than in any other pressing condition. Overall, we can conclude that the temperature threshold to achieve a bulk material varies significantly with the choice of applied pressure in the compression molding process. Low pressures shift the required temperature threshold to higher values while higher pressures reduce that temperature threshold. Yet the maximum tested pressure clearly is preventing homogenous sample formation as the error bars are significantly higher in these conditions. The maximum average modulus of 3.1 GPa was achieved at 35 kN and 140 °C. The minimum modulus was achieved at 7 kN and 60 °C.

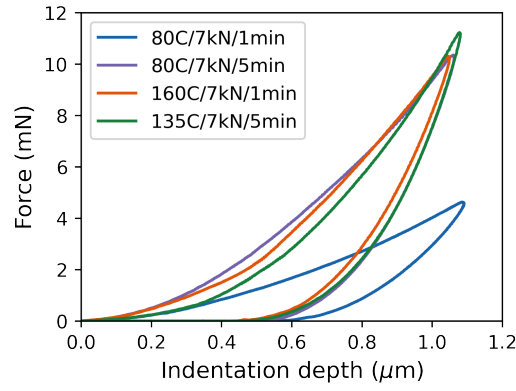


Figure 2.11: Representative loading and unloading curves procured from nanoindentation tests.

Representative loading and unloading curves from the nanoindentation measurements are shown in Figure 2.11. The reduced modulus was calculated using the slope of the unloading curve at maximum displacement along with the projected area of contact of the nanoindentation tip. This graph shows how poorly the material performs when the cells are being packed together without any plasticization occurring.

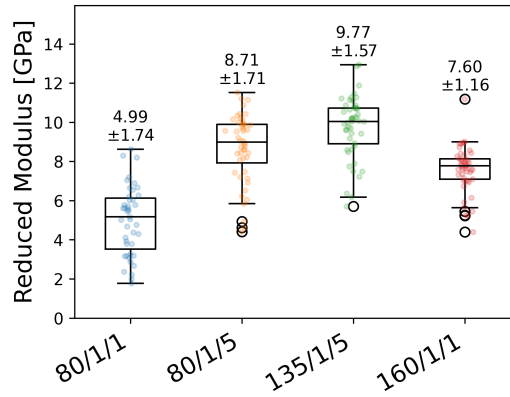


Figure 2.12: Nanoindentation data showing the reduced elastic modulus for various pressing conditions.

Figure 2.12 shows nanoindentation data for a set of samples deemed to be representative of the pressing samples, with the pressing conditions being listed as Temperature ($^{\circ}\text{C}$)/Hot Press Pressure (MPa)/Time (minutes). This figure shows the reduced modulus measured for each sample, with a “weak” sample at 80/1/1, two relatively strong samples in the middle, and a sample in the degradation temperature region at 160/1/1. The nanoindentation data corroborates the trend that is seen in Figure 2.13a and b, with stiffness increasing with

the intensity of the pressing conditions and dropping once the material starts to degrade.

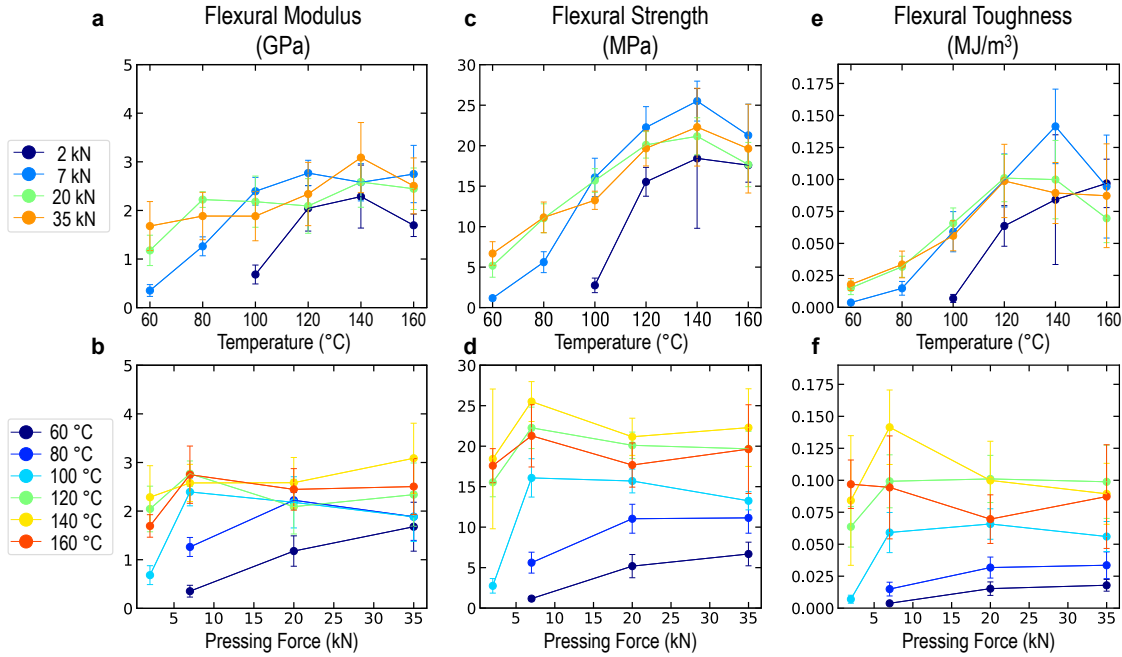


Figure 2.13: Effect of temperature and force on **a**, **b** flexural modulus, **c**, **d** flexural strength, and **e**, **f** flexural toughness, respectively.

The toughness of the specimens was similarly plotted against temperature and pressing force in Figure 2.13e and f. The toughness plots show almost the same trends as the strength plots. At the lowest pressing force of 2 kN, there is again a significant jump in toughness from 100 to 120 °C, from 0.01 ± 0.00 to 0.06 ± 0.02 MJ/m³, and it continues to increase up to 0.10 ± 0.02 MJ/m³. At 7 kN, the toughness at 60 °C is essentially zero, while it climbs up to 0.14 ± 0.03 MJ/m³ at 140 °C. It then sharply decreases to 0.09 ± 0.04 MJ/m³ at 160 °C. At 20 kN, the toughness increases from 0.02 ± 0.01 MJ/m³ at 60 °C to 0.10 ± 0.02 MJ/m³ at 120 °C, and decreases to 0.07 ± 0.02 MJ/m³ at 160 °C. At 35 kN, the toughness increases from 0.02 ± 0.00 MJ/m³ at 60 °C to 0.10 ± 0.03 MJ/m³ at 120 °C, at which point it reaches a plateau.

Overall, our mechanical tests show a strong dependency of the flexural strength of the produced bioplastics on the pressing temperature, with variations between the weakest bioplastic pressed at 60 °C and strongest at 140 °C (both pressed at $F = 7$ kN; pressure of 14.9 MPa) as high as 2080%. We further characterized the compressive mechanical properties of the optimally-pressed spirulina bioplastics. Interestingly, compression tests

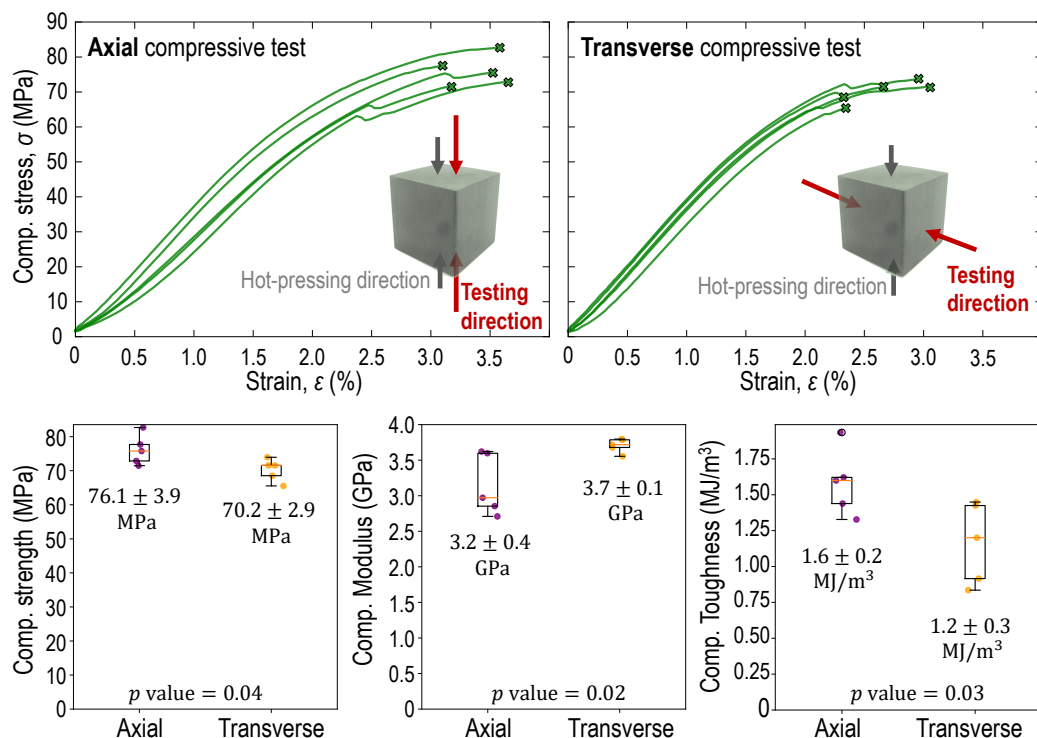


Figure 2.14: Compression tests in the axial and transverse directions with respect to the hot-pressing direction.

(Figure 2.14) reveal a nearly isotropic behavior.

Compressive strength was measured at 76.1 ± 3.9 MPa in the axial direction (with respect to the hot-pressing direction), and 70.2 ± 2.9 MPa in the transverse direction (7.7% difference). We attribute this small but statistically significant difference (p -value 0.04) to the fact that during the fabrication process, the compaction and binding of the bioplastics are initiated along the hot-pressing direction, which enhances the mechanical properties in that direction, before a continuous matrix is formed.

2.2.3 Morphological Changes

Along with the mechanical property changes, morphological changes were observed at the microscopic level. As stated before, the spirulina starts as particles of dried out, deceased spirulina cells. With weak or no self-bonding, the material ends up looking as shown in Figure 2.15. Parts of cells are still visible, and there are voids present in the final material. These voids are due to a lack of plasticization, but it is unclear if they are produced from the fracture mechanics of the material or from the poor packing of the powder at these low

pressing conditions.

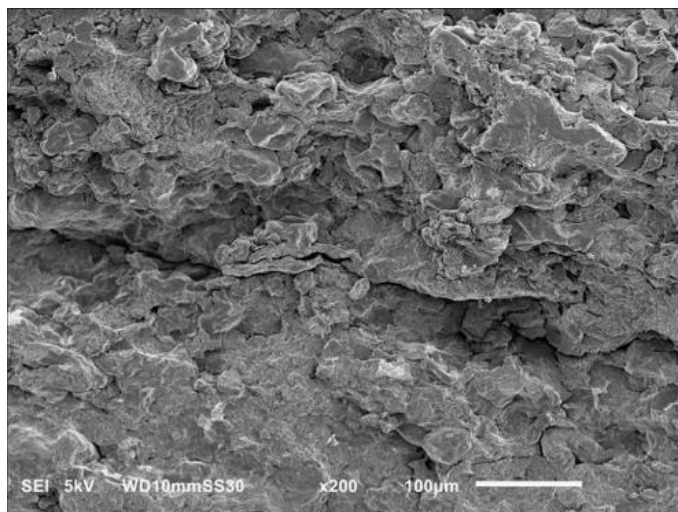


Figure 2.15: SEM image of a poorly self-bonded spirulina sample.

In contrast, Figure 2.16 shows the fracture surface of a fully plasticized sample at the same magnification. The sample appears smoothed out, and there are no individual cells visible. Much smaller cracks are visible, most likely from the fracturing rather than for powder-packing reasons. From Figure 2.15 to Figure 2.16, the material has completely changed its micro-morphology.

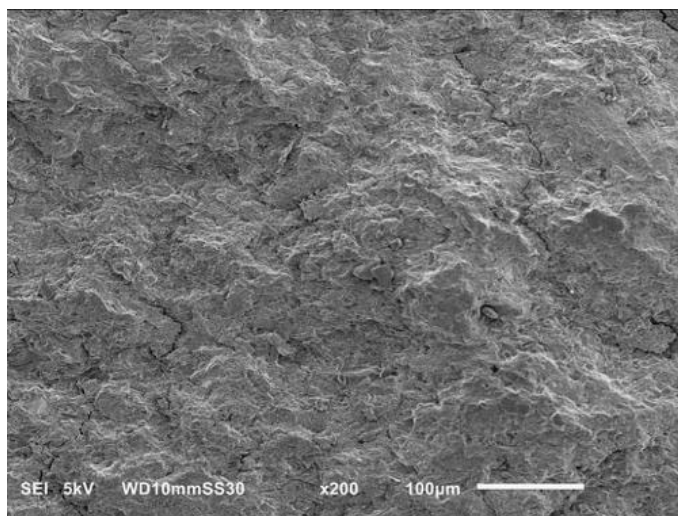


Figure 2.16: SEM image of a fully self-bonded spirulina sample.

The changes due to hot pressing can be observed on the macro scale as well. Figure 2.17a shows a group of poorly self-bonded specimens, where the properties come from powder packing rather than a change in the material. The pieces shed powder just from touching

or moving them, and they fall apart due to normal handling. In comparison, Figure 2.17b shows a well bonded specimen, which is smooth and reflects light with a polished surface. During tests where time was variable and temperature was extreme, short pressing times led to bubbles forming on the surface of the specimen, as shown in Figure 2.17c. This behavior is most likely due to the top layer of the specimen self-bonding while the underlying powder is still evaporating out humidity, leading to bubbles forming in the self-bonded layer.

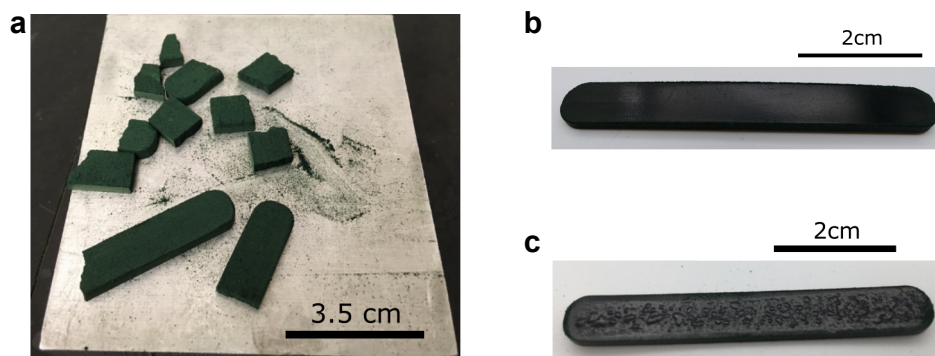


Figure 2.17: **a**, Poorly-bonded spirulina specimens. **b**, Well-bonded spirulina specimens. **c**, Spirulina specimen with bubbles on surface.

Energy dispersive X-ray spectroscopy (EDS) spectra, presented in Figure 2.18, show that nitrogen is evenly dispersed throughout the matrix of the bioplastic. This indicates that there is no phase separation or aggregation of protein, the dominant component by weight in our spirulina. The morphological uniformity ultimately endows the bioplastic with the remarkable stiffness and strength measured here.

Optical profilometry tests on the bioplastics pressed at 60 versus 140 °C (Figure 2.19), corroborate the microscopic observations and show that the well-bonded sample has a smoother surface with a root mean square height $S_q = 430$ nm, while for the poorly bonded sample, $S_q = 3.3$ μm . The profilometry maps also reveal the cell outlines on the surface of the weakest sample, in agreement with the SEM observations, while no cell outlines are seen on the strongest sample.

The smoothness of the strongest bioplastics is advantageous when producing faithful imprints of fine details through the use of positive molds. The roughness of the fracture surface of the weakest and strongest samples was also assessed using optical profilometry (presented in Figure 2.20).

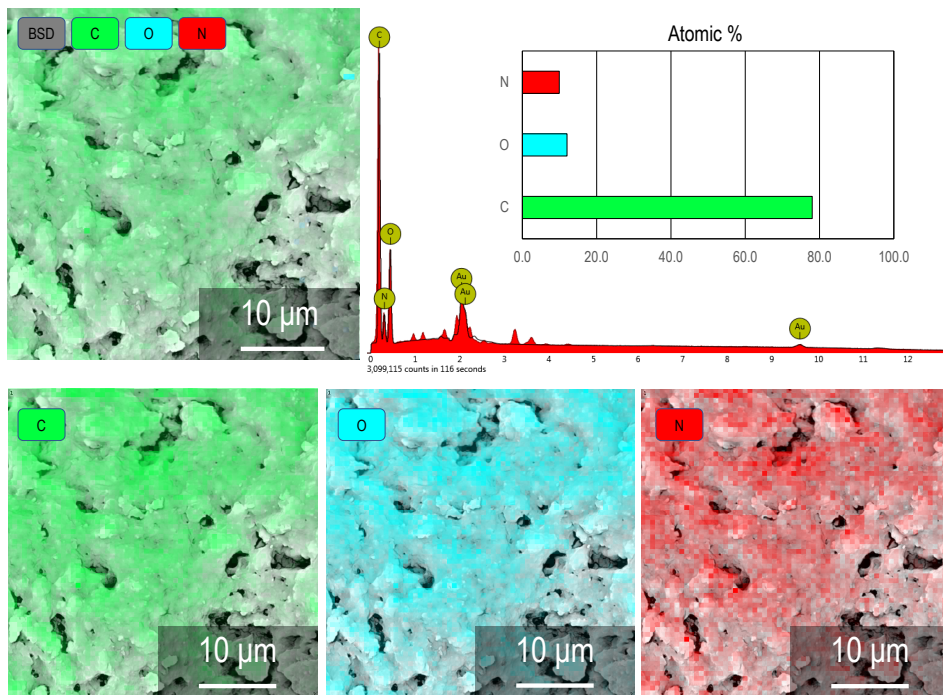


Figure 2.18: Energy dispersive X-ray spectroscopy (EDS) of a well-bonded spirulina bioplastic.

As expected, the well-bonded bioplastic resulted in a smoother surface ($S_q = 3.214 \mu\text{m}$) than the weakly-bonded samples ($S_q = 8.978 \mu\text{m}$). The mean square height of the weakly-bonded fracture surface is on the order of the spirulina cell dimensions, revealing once more that this sample corresponds to a compacted powder, whereas the well-bonded conditions enable the cells to fuse together.

We have presented clear effects of hot pressing on the mechanical and morphological properties of the bioplastics, and we will explore the molecular interactions that led to these changes in the next section.

2.2.4 Bonding Changes

To further understand the changes in bonding that led to the observable changes in the mechanical and morphological properties of our bioplastics, we conducted Fourier Transform Infrared (IR) spectroscopy and X-Ray Photoelectron Spectroscopy (XPS) on the spirulina powder and weakest and strongest samples (pressed at 60 and 140 °C, respectively), presented in Figure 2.22. Complete spectra and assignment of the IR peaks are presented below. (Figure 2.21 and Table 2.4).

Peak (cm ⁻¹)	Attribution	Source
3000-3500	$\nu(\text{O-H})$ with influence from inter- and intra-molecular hydrogen bonding	Ref. ¹⁶⁹
3270-3280	Amide A – resonance of $\nu(\text{N-H})$ stretch in proteins	Ref. ¹⁷⁰
3070-3080	Amide B - Resonance of $\nu(\text{N-H})$ stretch in proteins	Ref. ¹⁷⁰
2957, 2923, 2873, 2852	Aliphatic saturated C-H with different vibrational modes; $\nu_s(\text{CH}_3)$, $\nu_{as}(\text{CH}_3)$, $\nu_s(\text{CH}_2)$, and $\nu_{as}(\text{CH}_2)$, fatty acids, proteins, and carbohydrates	Ref. ¹⁷⁰
1735	Weak shoulder, $\nu(\text{C=O})$ stretching in fatty acids and peptides	Ref. ^{170, 171}
1654-1656	$\nu(\text{C=O})$ stretching amide I α -helical structures of proteins	Ref. ¹⁷⁰
1620-1640	$\nu(\text{C=O})$ stretching amide I β -sheet structures	Ref. ¹⁷⁰
1530-1540	$\nu(\text{C=O})$ stretching amide I β structures	Ref. ^{170, 171}
1451-1455	$\delta(\text{CH}_3)$ out of plane bending, $\delta(\text{CH}_2)$ in the lipid bilayer	Ref. ^{170–172}
1386	$\delta(\text{CH}_3)$ bending of fatty acids and amino acids; $\nu_s(\text{COO}^-)$ symmetric stretching	Ref. ^{170, 172}
1230-1237	Amide III ($\nu(\text{C-N})$, (N-H) bending); $\nu_{as}(\text{P=O})$ Nucleic acids, phosphoryl group	Ref. ^{170, 171, 173}
1150	$\nu(\text{C-O-C})$ in polysaccharides	Ref. ¹⁷⁴
1077	$\nu(\text{C-O})$ stretching of polysaccharides, at C6 in glucose rings	Ref. ^{170, 172}
1026	$\nu(\text{O=S=O})$ sulfate; $\nu(\text{P=O})$ nucleic acid, $\nu(\text{C-O})$ in polysaccharides	Ref. ¹⁷¹

Table 2.4: Spirulina FTIR Band Attributions

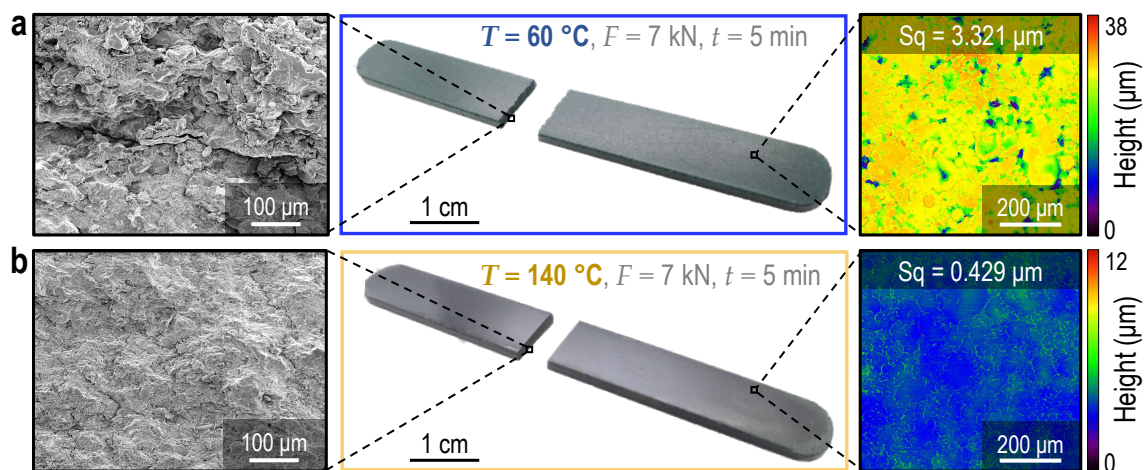


Figure 2.19: Scanning electron microscopy (SEM) images (left) and optical profilometry (right) of the weakest spirulina sample (top line) compared to the best spirulina sample (bottom line).

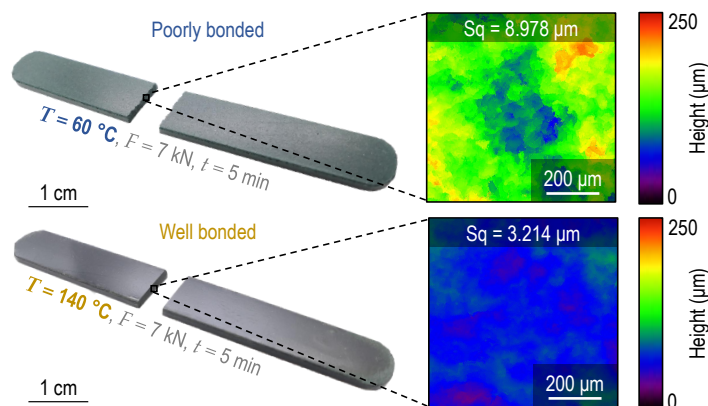


Figure 2.20: Surface profilometry profiles of the fracture surface of poorly and well bonded unprocessed spirulina bioplastics

The infrared spectra (Figure 2.22a) reveal significant differences between the spirulina powder and the bioplastic pressed at 140 °C. Thermomechanical processing at those conditions causes the Amide I, II, and III protein bands of spirulina to shift towards, and increase intensity at, lower wavenumbers. Most notably, the intensity of the Amide I band (1600-1700 cm^{-1}) of pressed spirulina increases significantly at 1623 cm^{-1} . The distinctive double peak formed in the strongest sample contrasts the broad Amide I band in spirulina powder centered at 1635 cm^{-1} . The Amide II and III bands also shift from 1539 to 1533 cm^{-1} and 1237 to 1230 cm^{-1} , respectively. Together, these transitions indicate the formation of β -sheet conformations in spirulina proteins during pressing^{175–177}. In a

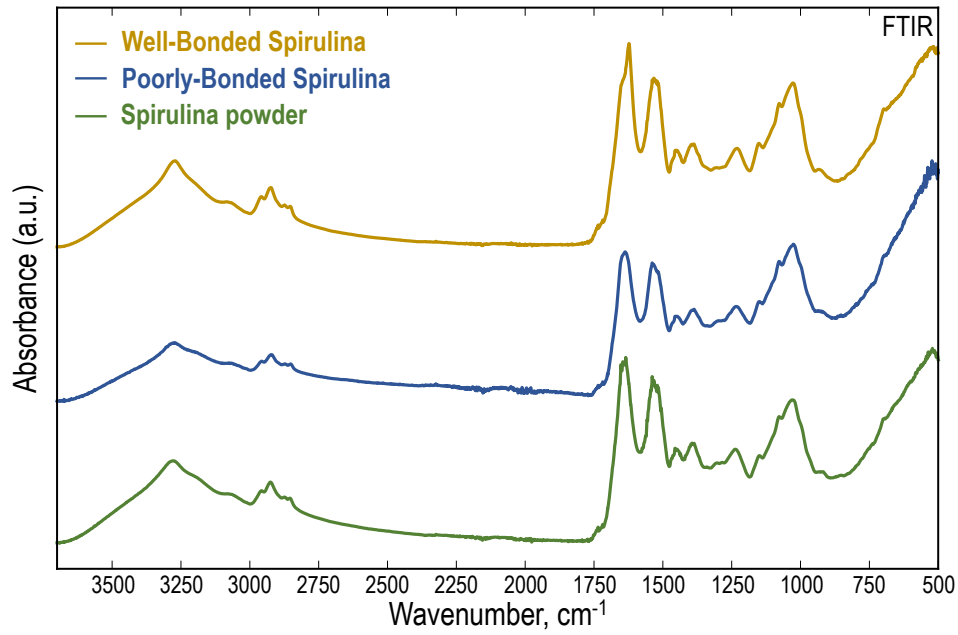


Figure 2.21: Fourier transform infrared (FTIR) spectra of as-received spirulina powder and hot-pressed spirulina bioplastics. Poorly-bonded samples were pressed at $T = 60\text{ }^{\circ}\text{C}$, $F = 7\text{ kN}$, $t = 5\text{ min}$ and well-bonded bioplastics at $T = 140\text{ }^{\circ}\text{C}$, $F = 7\text{ kN}$, $t = 5\text{ min}$.

fully protein-based system, Guo et al. showed that regenerated silk generated up to 40% β sheet content at higher temperatures of compression molding, compared to only 5-15% at lower temperatures. They found that at temperatures about $125\text{ }^{\circ}\text{C}$, strongly bound water is removed from the system and the silk fibroin chains are given enough mobility to form nanocrystalline β sheet configurations¹³⁵. These patent shifts observed in the Amide bands of spirulina pressed at $140\text{ }^{\circ}\text{C}$ are not observed after pressing at $60\text{ }^{\circ}\text{C}$. While there is a lesser shift in the Amide III band (from 1237 to 1234 cm^{-1}), the shapes and locations of the Amide I and II bands remain unchanged after hot-pressing using more mild conditions. This suggests that the protein transformation experienced during processing at $140\text{ }^{\circ}\text{C}$ does not occur for the pressing at $60\text{ }^{\circ}\text{C}$. β -sheet conformations are associated with higher strength and stiffness in proteins than α -helices^{178,179}. Therefore, the IR observations of an increased amount of β -sheets in the protein-rich spirulina matrix upon thermomechanical processing at a higher temperature ($140\text{ }^{\circ}\text{C}$) support the improved mechanical properties compared to the sample pressed at $60\text{ }^{\circ}\text{C}$.

Additionally, the IR spectra reveal a redshift in the O-H band of spirulina (3276 cm^{-1}) after pressing at both $60\text{ }^{\circ}\text{C}$ (3274 cm^{-1}) and $140\text{ }^{\circ}\text{C}$ (3271 cm^{-1}). Shifts in the O-H peak

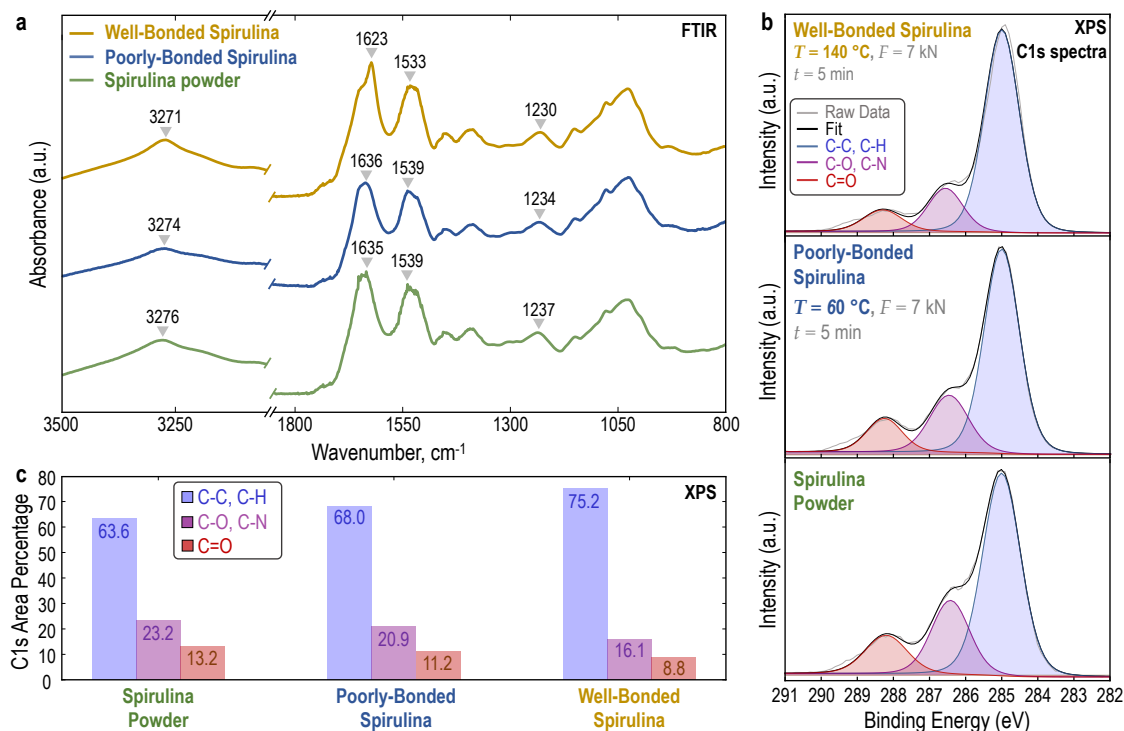


Figure 2.22: **Bond analysis of pure spirulina bioplastics.** **a**, Infrared spectroscopy of spirulina powder, poorly-bonded bioplastic (60 °C, 7 kN, 5 min), and well-bonded bioplastic (140 °C, 7 kN, 5 min). **b**, Area percentage numbers for the C-C/C-H, C-O/C-N, and C=O peaks as deconvoluted from the C1s spectra. **c**, C1s spectra from XPS for the spirulina powder, poorly-bonded bioplastic, and well-bonded bioplastic. Includes deconvoluted peaks corresponding to the C-C and C-H bonds, C-O and C-N bonds, and C=O bonds.

indicate changes in hydrogen bonding¹⁸⁰. The slight shift for the 60 °C pressed spirulina, for which significant protein conformational transformation does not occur, may indicate an increase in intermolecular hydrogen bonding that is unrelated to the formation of beta sheets. Therefore, this result suggests that the samples formed at the low-temperature condition are held together via intermolecular hydrogen bonding between the different biopolymers present in the spirulina cell walls and protoplasm, which are typically rich in surface hydroxyl groups^{181,182}. Other types of intermolecular interactions such as biopolymer chain entanglement and Van der Waals interactions may also contribute to the bonding of the samples pressed at lower temperatures. The more significant redshift of the O-H bond in the absorption spectrum of the sample pressed at 140 °C indicates more intermolecular hydrogen bonding in that sample. In this case, increased intermolecular hydrogen bonding can be attributed to both enhanced hydrogen bonding between biopolymers in the spirulina

and the transition from α -helices (intramolecular connections) to β -sheets (intermolecular connections).

The deconvolution of the collected XPS spectra (Figure 2.22b,c) reveals further changes in the bonding motifs of our materials. We observe a significant decrease in the relative amount of C=O, C-O, and C-N bonds in the hot-pressed materials, in comparison to the unpressed powder. Specifically, compared to the raw spirulina powder, the weakest bioplastic has 15% fewer C=O and 10% fewer C-O/C-N bonds while the strongest bioplastic has 33% fewer C=O and 31% fewer C-O/C-N bonds. Both pressed samples have higher amounts of C-C/C-H groups compared to the unpressed powder (7 and 18% higher respectively for the 60 and 140 °C). The spirulina cells used in this study are comprised of 54.2-63.1% protein as measured by elemental analysis (CHN), 15% saccharidic carbohydrates and 15% acid-soluble phenolics as determined by HPLC⁹⁹, 1.9-7.8% lipids¹⁸³⁻¹⁸⁵, and 1-10% PHA^{186,187}. We first consider the dominant biopolymer components (proteins, glucose-based carbohydrates and phenolics)⁹⁹ to understand the possible changes in bonding. Proteins are rich in carbonyl groups in the peptide chains, some of which are exposed in the folds, turns, and chain ends of the proteins. Carbohydrates on the other hand are rich in pendant C-O groups. These groups are also present in the main sugar chain but the side groups are more reactive¹⁸⁸. Aromatic compounds in spirulina also offer a variety of functional groups including C=O and C-O pendant groups^{182,188}. We hypothesize that heat and pressure facilitate reactions of the C=O and C-O bonds at chain ends and pendant sites which result in the increased amount of C-C bonds measured in the spectra of hot-pressed samples. Therefore, we observe distinct changes in the covalent bond makeup of the spirulina samples upon hot pressing in addition to the hydrogen bonding suggested by the IR spectra. Our data collectively suggest that the processing of spirulina powder with heat and pressure leads to changes in covalent bonding and enhances the secondary interactions between the biopolymers within the biomatter in addition to causing conformational changes (β -sheet formation) in the protein matrix. These changes together support the measured increases in the mechanical properties as the biomatter is processed at higher temperatures. The presence of PHA in spirulina may also contribute

to the bonding of the bioplastics, but because of its relatively low concentration^{186,187} and high melting point^{72,189}, its contributions are likely minimal compared to those of the other biopolymers discussed above.

2.2.5 Mechanical Recyclability

Aiming to determine the ability of the bioplastics to be re-used and re-formed, we mechanically disintegrated a self-bonded pure spirulina bioplastic through grinding to produce a powder. The powder was subsequently incubated in ambient conditions for 24 hours before re-pressing it at the same hot-pressing conditions. The flexural strength of the regenerated bioplastics is compared to the original strength (generation 0) in 2.23b, showing gradual loss in strength over the course of six generations (starting at a strength of 29.5 ± 2.4 MPa at generation 0, down to 22.2 ± 4.6 MPa at generation 5). The stiffness remained consistent through the regeneration cycles, ranging from 3.5 ± 0.7 GPa to 3.2 ± 0.5 GPa at the fourth regeneration.

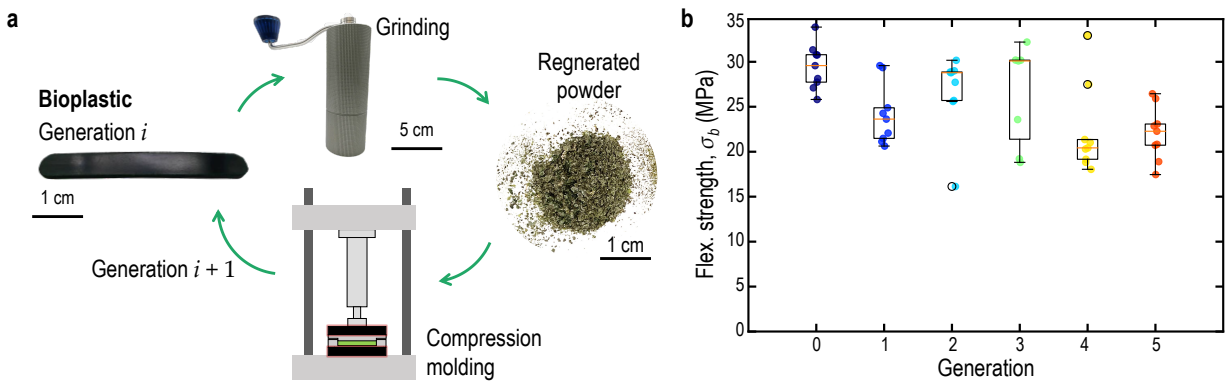


Figure 2.23: **a**, Spirulina bioplastics can be recycled by grinding and re-pressing. **b**, Flexural strength values for regenerated spirulina samples.

We note that a reduction in the mechanical properties due to thermo-oxidative and thermo-mechanical degradation is also seen in synthetic recyclable thermoplastics like polyolefins and polyesters, which rely on the introduction of stabilizers and additives to ensure performance recovery after successive reprocessing cycles¹⁹⁰. Overall, the noteworthy thermoplastic behavior and recyclability of our bioplastics suggest their applicability in areas where conventional thermoplastics are currently used.

2.2.6 Effects of Sonication

To expand the mechanical property space of the spirulina bioplastics, we investigated mechanical pre-treatments of the cells. We hypothesized that mechanically pre-treating the spirulina cells to dissociate them would improve bonding, as a higher surface area would be available for interactions during the pressing step. We sonicated the as-received cells in water (see Section 2.1), as ultrasonication has been proven to cause cell disruption in spirulina cells^{191,192}. SEM images confirm that sonication leads to cell dissociation and, as a result, to a substantial particle size reduction from $28.3 \pm 16.6 \mu\text{m}$ to $5.5 \pm 3.5 \mu\text{m}$ (Figure 2.24). Following this step, we freeze-dried and ground up the sonicated spirulina before pressing it at $140^\circ\text{C}/35 \text{ kN}/5 \text{ min}$.

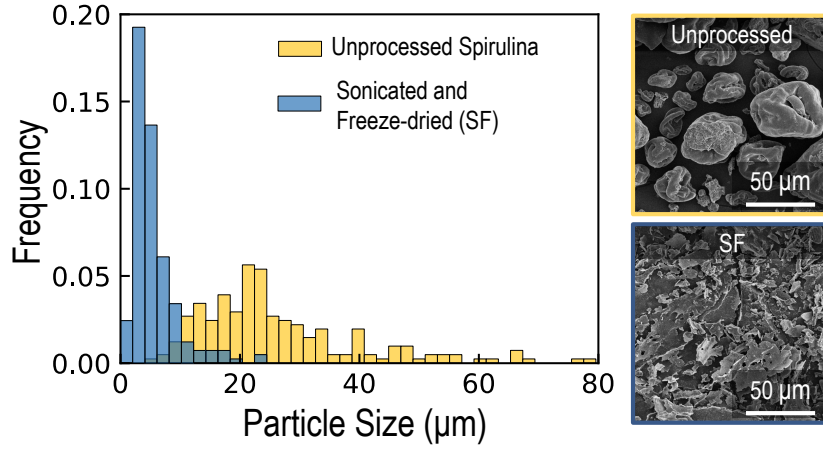


Figure 2.24: Particle size distribution of unprocessed and sonicated (SF) spirulina cells and corresponding SEM images.

The morphological analyses through SEM and optical profilometry reveal that the cell disruption via sonication further improves the homogeneity of the matrix after hot-pressing (Figure 2.25) leading to the smoothest surface with $S_q = 117 \pm 60 \text{ nm}$.

Figure 2.26 shows representative three-point bend stress-strain curves of sonicated spirulina bioplastic compared to the non-sonicated equivalent (solid green and purple lines, respectively).

On average, the sonicated spirulina has a bending strength of $35.1 \pm 4.5 \text{ MPa}$, corresponding to a 38% increase compared to the strongest unprocessed spirulina sample, while the modulus is also increased by 58% compared to that sample. Therefore, mechanical

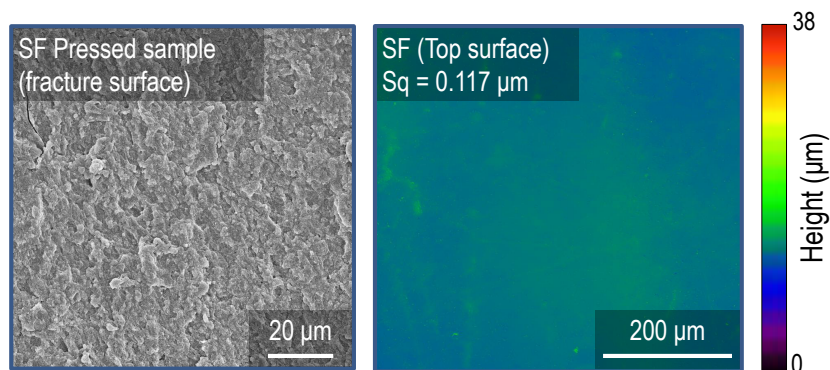


Figure 2.25: SEM (left) and optical profilometry (right) images of the spirulina bioplastics created from sonicated cells.

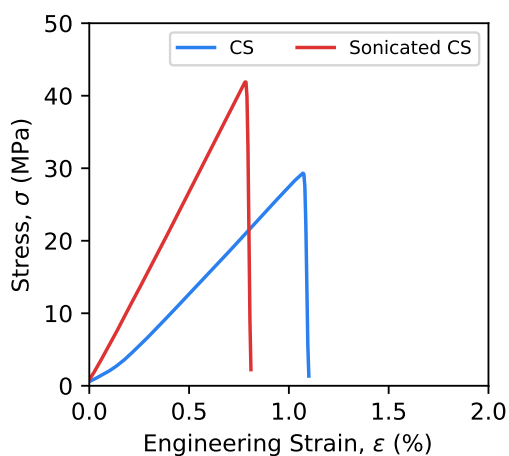


Figure 2.26: Representative stress-strain curves of sonicated spirulina bioplastics compared to un-treated spirulina bioplastics.

pre-treatment alone can be an effective strategy to improve the attainable properties of pure spirulina bioplastics.

2.3. Expanding Mechanical Properties of Spirulina Bioplastics

2.3.1 Addition of Plasticizer

As an alternative strategy to improve the toughness and flexibility of our bioplastics and expand their processability from compression molding to extrusion, we examined the use of plasticizers. Previous literature reports that glycerol can effectively plasticize spirulina, allowing it to be processed in an extruder¹⁰⁵. However, the reported mechanical properties are

lower than the ones achieved in our work. In the absence of plasticizer, their pure microalgae samples attain strengths in the range of 2-6 MPa (compared to our maximum strength of unprocessed hot-pressed spirulina of 26 MPa), while in the presence of 25% glycerol, they report strengths between 1 and 2 MPa (we report strength 4-10 MPa for hot-pressed spirulina in the presence of plasticizer). Given our observations that higher temperatures and pressures are required for the transformation of spirulina to a fully bonded matrix, we chose to use sorbitol, another polyol derived from biomass, as a plasticizer instead of glycerol, due to its higher melting point and solid form at room temperature that allows easy blending with the spirulina powder. We prepared composites with 0-30 wt.% sorbitol and pressed them to 80 °C at 2 kN. The hot pressing conditions were limited by the low melting temperature of sorbitol (90 °C) and the tendency for the composites to melt and flow out of the mold. The concentration of sorbitol was kept at or below 30% for the same reason. As discussed in the previous section, the pure spirulina processed using these mild conditions has an un-bonded, packed-powder morphology, with little strength or toughness as shown in Figure 2.27c. As sorbitol concentration increases, a gradual increase in strength and toughness is observed. The 5 and 10 wt.% sorbitol bioplastics have a 41.4% and 90.5% increased modulus, 45.9% and 136.4% increased strength, and 100% and 300% increased toughness compared to the neat spirulina, as shown in Figure 2.27d and e. At 30 wt.% sorbitol, the strength reaches 10.2 ± 1.6 MPa and the toughness is 0.16 ± 0.05 MJ/m³, marking increases of 3 and 16 times, respectively, compared to the pure spirulina control. The highest strain-to-break values (2.8%) are achieved at the maximum sorbitol concentration of 30 wt.%, showing a 2.4-fold increase over the pure spirulina. Figure 2.27b further demonstrates that the substantial increase in the extensibility of the bioplastics produced using the maximum sorbitol content allows an extruded filament to bend much more than the control material. Based on our mechanical tests, we conclude that the introduction of sorbitol at concentrations up to 30 wt.% promotes larger deformation prior to failure and improves the strength and toughness of spirulina.

The SEM images in Figure 2.27c reveal the effects of sorbitol on the micromorphology of the bioplastics. The spirulina sample with no sorbitol is comprised of weakly-bonded, in-

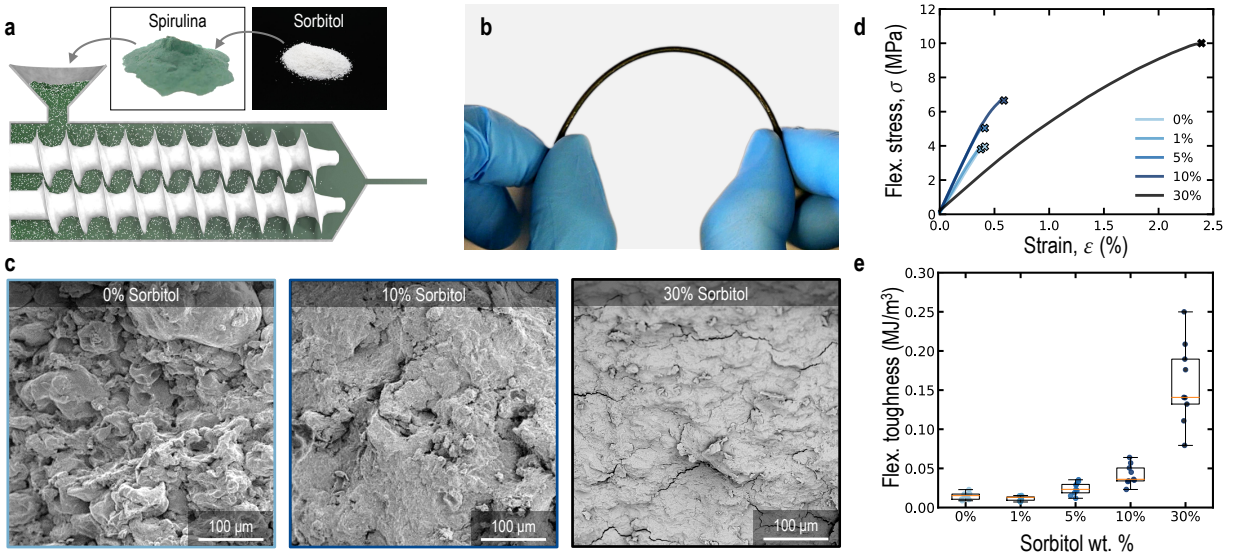


Figure 2.27: **Sorbitol as a plasticizer for spirulina.** **a**, Schematic for the processability of spirulina/sorbitol mixtures in a twin-screw extruder. **b**, Extruded filament containing 30 wt.% sorbitol demonstrating flexibility. **c**, SEM fracture surface images revealing the micromorphology of the spirulina bioplastics containing 0%, 10%, and 30% sorbitol. **d**, Representative stress-strain curves and **e**, boxplots for flexural toughness of the spirulina/sorbitol bioplastics.

tact, packed spirulina cells, as discussed previously. When sorbitol is introduced in the formulation, it melts and forms a cohesive matrix surrounding the cells, which are ultimately bonded together without being disrupted. Our imaging and mechanical results collectively suggest that intact spirulina cells can be efficiently bonded through the surrounding sorbitol matrix, which drives significant improvements in the strength, extensibility, and toughness of the bioplastics.

2.3.2 Adding Nanoclay and Bacterial Cellulose

In an attempt to further improve the mechanical properties of our bioplastics, we incorporated nanoclay platelets and bacterial cellulose (BC) sheets as nanofillers in the spirulina matrix. The incorporation of nanofillers is a common approach to enhance the mechanical properties of conventional polymers^{193,194}. We selected nanoclay and BC as case studies because of their exceptional mechanical properties, hydrophilic nature, and compatibility with our biopolymer matrix, as well as for their distinctly different morphology and aspect ratios^{166,195}. Specifically, the layered conformation and terminal oxygen planes in

the tetrahedral structure of montmorillonite (MMT), our nanoclay of choice, would allow enhanced surface interactions and hydrogen bonding with the protein- and carbohydrate-based spirulina matrix⁹⁹. We note that sonication of the nanoclay and spirulina in water are necessary to promote filler dispersion and enhance interactions by dissociating the cells.

Indeed, upon freeze-drying and hot pressing the nanocomposite bioplastics, we observe that a 5 wt.% nanoclay concentration leads to an improvement of the average flexural strength from 35.1 ± 4.5 to 57.2 ± 7.4 MPa (63% increase), a increase in work to fracture from 0.15 ± 0.03 to 0.29 ± 0.06 MJ/m³ (93% increase), and an increase in stiffness from 3.9 ± 0.7 to 5.3 ± 0.3 GPa (36% increase) over sonicated and freeze-dried spirulina without the nanoclay (Figure 2.28f, solid blue line).

SEM images of the nanoclay nanocomposites show no observable change in the micromorphology as compared to the neat sonicated spirulina (Figure 2.28d), suggesting sufficient dispersion of the nanoclay in the matrix. No visible aggregation was noted and bending failure occurred at the uniform matrix rather than in any filler-rich area. While further research will be necessary to thoroughly characterize the bonding between MMT and the matrix, the results show a significant increase in strength, stiffness, and work to fracture at concentrations as low as 5 wt.% of MMT.

As a fiber nano-reinforcement for our spirulina matrix, we selected BC fibers due to their exceptional mechanical properties and high aspect ratio which, together with their abundant surface hydroxyl groups, would enable the formation of a strong network of interactions with the spirulina matrix biopolymers. In this example, we choose to use BC in the form of a sheet, as grown from a bacterial culture¹⁶⁶, and create layered nanocomposites instead of isotropic ones. The ultra-long cellulose fibers synthesized by the cultured bacteria form a strong multilayered structure that utilizes the vast network of hydrogen bonding and physical interlocking of the intra- and inter-planar fibrils to achieve in-plane tensile strengths as high as 300 MPa and moduli up to 20 GPa¹⁹⁶. As reported by Fredricks et al., the bacterial cellulose used in the present study had a tensile strength of ~ 150 MPa and tensile modulus of ~ 10 GPa¹⁶⁶.

Aiming to capitalize on the high strength and stiffness of the self-bonded cellulose sheets

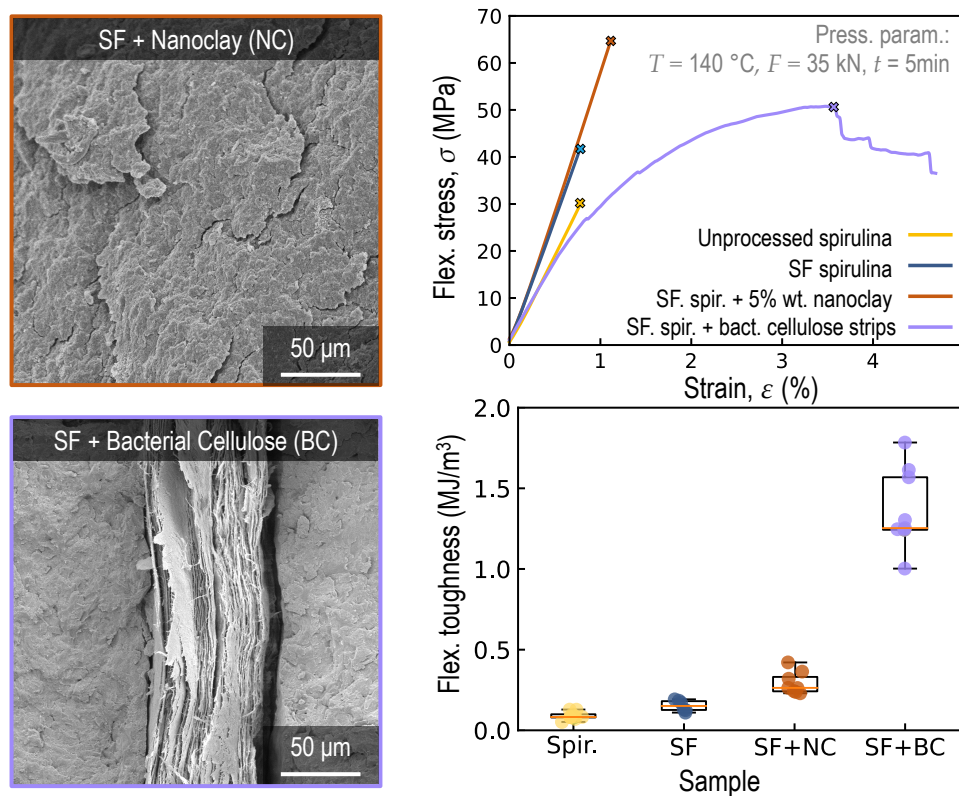


Figure 2.28: SEM images of the fracture surfaces of bioplastic nanocomposites containing nanoclay (NC, top-left) and bacterial cellulose (BC, bottom-left). Comparison of representative stress-strain curves of pure spirulina, sonicated spirulina, sonicated spirulina with NC, and sonicated spirulina with BC (top-right). Effects of sonication and fillers on the toughness of the generated bioplastics (bottom-right).

in their native (as-synthesized) state, we created a lamellar structure in which single layers of BC sheets were sandwiched between spirulina powder, before subjecting the composite to hot-pressing. The bending tests conducted in the plane normal to the BC sheets reveal that the addition of BC causes significant changes in the mechanical behavior of spirulina, as reflected in the representative stress-strain curve of Figure 2.28f (solid yellow line). While the stiffness of the composite is less than that of the neat spirulina, significant strengthening and toughening are conferred by the presence of BC sheets (Figure 2.28f, g). Specifically, the BC nanocomposite has an average toughness of $1.4 \pm 0.2\text{ MJ/m}^3$, which is $15\times$ higher than the pure, untreated spirulina processed at the same conditions, while the strength reaches values as high as $42.25 \pm 9.06\text{ MPa}$, 98.5% higher than pure spirulina. The SEM image of the fracture surface shown in Figure 2.28e reveals that failure occurs at the interface between the algal matrix and the cellulose sheets, suggesting that the

bonding between cellulose and algae is weaker than the matrix or the cellulose itself. Still, the positive impact of the BC sheet addition on the mechanical properties is evident, and future work could further improve composite performance by improving the interfacial interactions.

2.4. Comparison to Commodity Plastics

Having characterized the mechanical properties of spirulina bioplastics and their enhancement using conventional polymer processing methods, we now compare and contrast our bioplastics with common plastics. Specifically, we focus on mechanical performance, flammability, ability to be patterned, recyclability, and compostability.

2.4.1 Mechanical Properties

In Figure 2.29a we present the mechanical property space of spirulina-based bioplastics covered by the discussed approaches. Elastic moduli between $E = 0.4$ and 5.3 GPa and flexural strengths from $\sigma = 1.17$ to 57.16 MPa place our bioplastics within the performance space of commodity plastics. As discussed above, our bioplastics have similar mechanical properties to PLA and drastically outperform previously reported results for bioplastics made from entire microalgae cells¹⁰⁵ and TPS. In the family of the biodegradable and bio-sourced PHAs and their copolymers such as poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) also shows promising properties. Neat PHBV typically has an elastic modulus around 1 GPa and strength below 20 MPa¹⁹⁷. It should however be emphasized that, while showing encouraging results, PHA extraction involves complex processes that currently jeopardize its industrial scalability and limits its sustainability^{197–199}. Thermoplastic starch provides the advantage of being biodegradable and requires low-complexity extraction methods, but its elastic modulus and strengths are typically low compared to other plastics, with $E = 0.003$ –1 GPa and $\sigma = 0.2$ –6²⁰⁰. Finally, while some high-performance petroleum-derived polymers undoubtedly have more attractive mechanical properties, our bioplastics perform similarly to most consumer-based plastics such as polyethylene (PE: $E = 0.25$ –1.25 GPa, $\sigma = 10$ –32 MPa), polypropylene (PP: $E \sim 2$ GPa, $\sigma \sim 26$ MPa), polystyrene

(PS: $E \sim 3$ GPa, $\sigma \sim 34$ MPa), or Polyethylene terephthalate (PET: $E \sim 2.3$ GPa, $\sigma \sim 55$ MPa)²⁰¹.

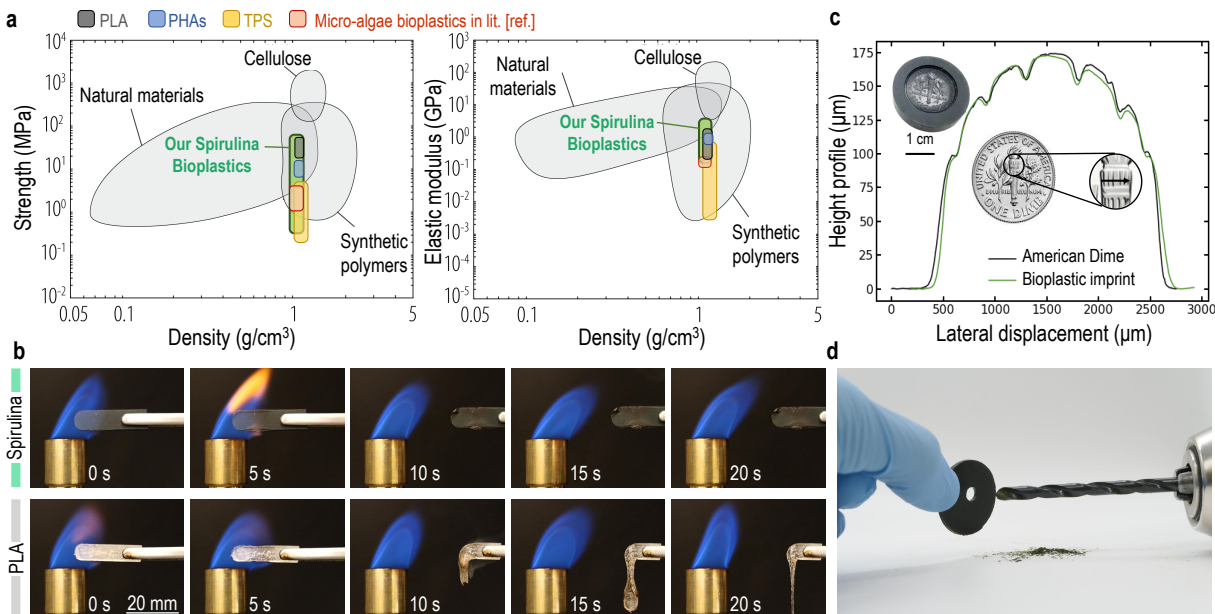


Figure 2.29: **Spirulina in the context of commodity plastics.** **a**, Ashby plots comparing the strength and moduli of the reported bioplastics with natural materials, synthetic plastics, and PLA. **b**, Flammability comparison of spirulina versus PLA. **c**, Contact profilometry of a portion of a dime and that same portion imprinted on a spirulina bioplastic. **d**, Machinability of spirulina bioplastics demonstrated using an electric drill.

2.4.2 Machinability

In Figure 2.29c we demonstrate that the plasticity of the spirulina bioplastics upon hot-pressing allows them to faithfully inherit imprints from positive molds. Nesting an American dime within a cylindrical mold filled with spirulina powder and subjecting it to hot pressing enabled successful transfer of the coin's design as an imprint on the spirulina surface. The surface profilometry results comparing the pattern of the coin and the imprint on the bioplastic surface show accurate patterning. The profilometer tip was run horizontally along the torch on the dime and the imprint on the spirulina, with the two curves having a root-mean-square deviation of $7.8 \mu\text{m}$. Further evidence for the workability of the bioplastics is presented in Figure 2.29d where basic machining is demonstrated using conventional tooling (a hand-held drill). These abilities suggest that as manufacturing with non-traditional materials becomes more commonplace, spirulina and other similar

biological matter feedstocks will be promising candidates as biodegradable polymers for commodity products.

2.4.3 Flammability

In terms of flammability, our bioplastics outperform the benchmark industrially compostable bioplastic, PLA, as shown in Figure 2.29b. After exposure to an open flame for 10 s, the spirulina bioplastic sample self-extinguished within less than 1 s and produced a char, while PLA combusted and melted leaving no solid residue.

2.5. End of life: Biodegradation Kinetics and Mechanisms

2.5.1 Biodegradability

Given that the vast majority of conventional plastic waste resides in the environment or accumulates in landfills⁹, a desirable end-of-life for our spirulina bioplastics is fast biodegradation. More specifically, the possibility for these bioplastics to degrade in soil (in a natural, aerobic environment) without the need for special conditions, such as those required for the industrial degradation of PLA, would enable end-of-life disposal in natural environments with minimal environmental impact.

Our first experiments will focus on aerobic degradation and the kinetics data will be obtained from following the mass loss over time. In Figure 2.30a, we present photographs of spirulina samples buried in soil for natural decomposition right after burying (day 1), and after approximately 16 weeks (111 days). The advanced biodegradation is visually observed from the blending in of the samples with soil on day 111.

To quantitatively assess this degradation rate, we measured the mass loss over time of spirulina bioplastics buried in soil, as compared to a positive control (banana peels) and a negative control (PLA). As reported in Figure 2.30b, the spirulina bioplastics degrade at a rate comparable to the positive control, with a rapid mass loss upon exposure to soil. Indeed, during the first 5 weeks of incubation in soil, both the positive control and our

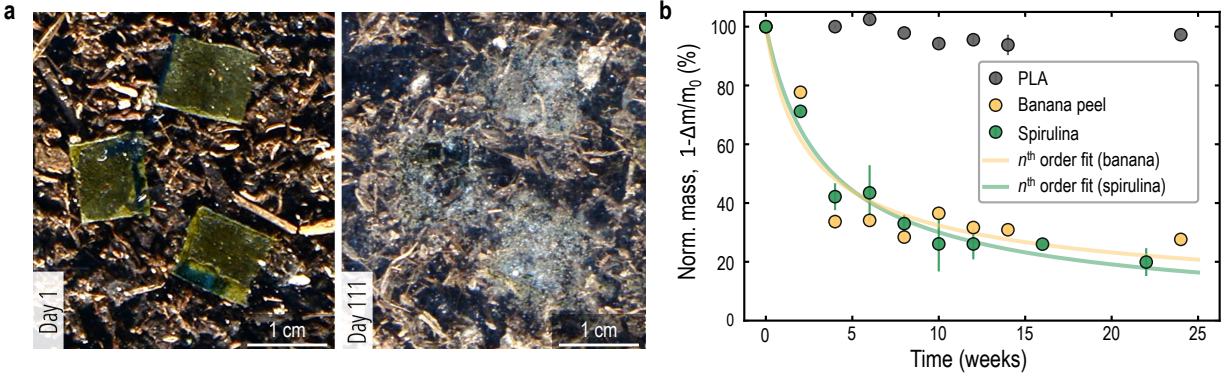


Figure 2.30: **a**, Photographs of the biodegradation of algal bioplastics in soil **b**, Mass loss plot comparing the biodegradation of spirulina bioplastics in soil against banana peel as a positive control and PLA as a negative control.

bioplastics lose approximately 60% of their dry mass and subsequently continue to slowly degrade. A mass loss of about 80% is achieved after 22 weeks, both for the bioplastics and the positive control. This type of decelerating degradation has been observed in many types of biodegradation tests (enzymatic, in water, in soil) for various biomass materials²⁰². Considering that decelerating degradation kinetics are often well described by reaction order models^{203,204}, we found that an n^{th} order reaction model of the type:

$$\frac{d\alpha}{dt} = k(1 - \alpha)^n, \quad (2.3)$$

where $\alpha = \Delta m/m_0$ corresponds to the degree of conversion with Δm the mass variation and m_0 the initial mass of the buried sample, gives a reasonable fit of our data. A least-square fitting provided values of $n = 2.89, k = 0.29$ and $n = 2.28, k = 0.39$ for banana and spirulina, respectively, with corresponding root-mean-square error values of 0.013 and 0.028. To the best of our knowledge, no prior investigation has focused on the degradation kinetics of spirulina in soil. One study reported a satisfactory first-order model ($n = 1$) for the anaerobic biodegradation of spirulina in the presence of sewage sludge²⁰⁵, but additional investigations will be necessary to further shine light on the soil-degradation reactions and kinetics of our bioplastics. Still, our results reveal the natural biodegradation capability of the spirulina bioplastics over the course of a few weeks.

2.5.2 Life Cycle Analysis

Analyzing the environmental impacts associated with the manufacturing process of the spirulina bioplastics offers valuable insights into the true potential of the suggested bioplastics class. Assuming that the environmental impact associated with hot-pressing spirulina powder into a functional part is similar to the impact of the molding step required to fabricate a part from traditional plastic pellets, we restrict our comparison to the cradle-to-factory-gate production of spirulina powder vs. traditional plastic pellets. Given that the growth of plants or algae takes up carbon from the atmosphere, their use as feedstocks in bioplastics enables the fabrication of carbon-neutral or carbon-negative materials. Upon growth, microalgae capture around 1.8 kg CO₂ per kg of dry biomass²⁰⁶, providing an attractive advantage over petroleum-derived polymers. It should be noted that the CO₂ emissions associated with fertilizers, heating, pumping, harvesting, and drying are highly dependent on the installations and therefore vary significantly across sources.

By using the global warming potential (GWP) values of the production of wet spirulina²⁰⁶ and the drying process of microalgae²⁰⁷, we estimate a GWP of -0.54 kg CO₂ / kg for our dry spirulina powder. While it is important to note that Tzachor et al.'s production method is not typical, it is an indication that newer and cleaner methods of microalgae production are possible. In a recent study by Beckstrom et al., the authors reported an overall net carbon credit of -0.315 kg CO₂ / kg of dry microalgae in their best-case models²⁰⁷. For comparison, the GWP of 1 kg of high-density polyethylene pellets was reported to be 1.9 kg CO₂ by PlasticsEurope²⁰⁸, while polypropylene was reported to have a GWP of 2.0 kg CO₂. In 2019, a cradle-to-gate study²⁰⁹ showed the GWP of Corbion PLA pellets to be 0.501 kg CO₂ / kg of PLA. The potential negative GWP of the spirulina bioplastics, their ability to be processed using conventional polymer manufacturing infrastructure, their impressive attainable mechanical properties, and their end-of-life fates, suggest that this material class represents a viable alternative to commodity plastics for a wide range of applications.

2.5.3 Conclusion

The design and fabrication of environmentally friendly materials must be investigated to mitigate the plastic pollution generated by non-degradable polymers. In this Chapter, we have successfully demonstrated the production of bulk, thermoformable plastics using an abundant, photosynthetic microorganism, spirulina. This wasteless transformation does not require additional binders or solvents and yields bioplastics with mechanical properties comparable to polystyrene (34 MPa tensile strength, 3 GPa flexural modulus, 1.6% elongation to break). We have shown further control of the mechanical properties by pre-processing the biomass through sonication and by introducing fillers, which allow us to expand the attainable bending strength by 124 %, the modulus by 71 % and the toughness by 900 %. We have reported the first step towards understanding the chemical transformations driving the macroscopic bonding upon hot-pressing, though further investigations will be required to elucidate the precise reaction mechanisms. These algae-based bioplastics are machinable and have remarkable shape fidelity when making imprints. They also show potential to be used as flame-safe materials as they self-extinguish and char almost immediately after exposure to an open flame. These bioplastics can be given a second life by grinding into powder for re-processing, or they can harmlessly return to the environment by degrading in soil. Given these attractive properties and the fact that spirulina is carbon negative, the proposed material family is appealing for packaging or consumer goods applications, which are currently mostly unsustainably sourced by petroleum-derived polymers.

3. Assessing the impacts of colloidal-state properties on the solid state of mixed-solubility carbohydrate binary networks

This chapter is based on the following published work:

Hareesh Iyer, Aban Mandal, Michael Holden, and Eleftheria Roumeli. Modifying bacterial cellulose dispersions with deep eutectic solvent and pectin to tune the properties of open-celled foams. *RSC Applied Polymers*, 3(2):407-419. Publisher: The Royal Society of Chemistry.

3.1. Materials and Methods

3.1.1 Materials

12 oz. kombucha SCOBYs were purchased from joshuatreekombucha.com. Gallon black tea bags were purchased from Lipton. Pectin from citrus peel, choline chloride, citric acid from citrus peel, and sodium hydroxide were purchased from Sigma-Aldrich.

3.1.2 Methods

Bacterial cellulose production

Tea media was produced by bringing 4 L of deionized (DI) water to a boil and steeping 4 teabags for 5 minutes. After steeping, the teabags were removed, and 800 g of sugar was dissolved into the tea solution. The solution was allowed to cool to below 35 °C. A Ninja

blender (model BL7730) with a blender cup assembly (sterilized with ethanol) was used to blend 4 SCOBYs in 5 second intervals over the course of 2 minutes. The blended SCOBYs were added to the 4 L of tea media before being placed into a glass fish tank measuring 60 cm in width, 30 cm in depth, and 40 cm in height. The tank was covered with a muslin cloth to protect the growth while also allowing for air exchange. The growth solution was left for 14 days before the pellicle was harvested and purified by soaking in 1 M NaOH for at least 24 hours. The pellicle was then immersed in DI water for rinsing. The water was drained and replenished until the pellicle turned white and exhibited a neutral pH.

BC dispersions were then made by blending 75 g of the purified BC pellicle with 200 ml of DI water for 3 minutes. 10 g of the dispersion was moisture analyzed using a VWR M-Series moisture analyzer to determine water content.

DES treatment

The citric acid-based DES was made by heating a 1:1 molar ratio of citric acid and choline chloride to 80 °C while constantly stirring until a homogenous liquid formed. The BC dispersion and DES were combined in a 1:15 ratio of BC:DES by mass. Approximately 1 L of boiling DI water was added, the beaker was covered with aluminum foil, and the mixture was heated to 105 °C for 2 hours on a hot plate with a magnetic stir bar. Once the DES treatment was done, the solution was cooled by adding cold DI water to the beaker. The solution was then vacuum filtered using a Whatman #2 filter. The resulting film was separated from the filter paper and blended for 3 minutes in DI water. This process of vacuum filtering and washing was repeated once more. The resulting dispersion was then moisture analyzed before being centrifuged down to 1 wt.% of BC in water.

Mixing composite dispersions

Pectin was added to DI water to make a 1 wt.% solution, and was stirred on a hot plate at 100 °C for 5 minutes to fully dissolve. The solution was allowed to cool to approximately 60 °C before being combined with the 1 wt.% CADES-BC dispersion in the desired ratio of the composite components.

Foam production

3-part cylindrical molds consisting of an open-ended cylinder and two end caps, were 3D printed using an Anycubic Photon SLA printer and their basic, clear resin. The interior dimensions of the cylinder were 15 mm in diameter and 15 mm in height. The molds were lightly coated with a Smooth-On universal mold release agent and carefully packed to ensure no air was trapped in the mold. With one cap removed, the filled molds were placed under a vacuum of 0.5 psi to degas the dispersion for 5 minutes. After degassing, the top caps were placed onto the molds and the samples were loaded into a freezer for 24 hours. The top and bottom caps were removed from the frozen samples and a razorblade was used to shave down the frozen material where it had expanded out of the mold. Finally, the frozen samples were placed on their sides such that the two ends were exposed to the atmosphere in a freeze dryer (Freezon 2.5 L, Labconco Corp) for 24 hours.

Scanning electron microscopy

Samples were sputter coated using a Quorum SC7620 with gold-palladium for 30 seconds. SEM imaging was performed on a JEOL JSM-6010 Plus and micrographs were collected at over a range of 25x to 3000x magnification.

Zeta potential

The zeta potential was measured by Dynamic Light Scattering using a Malvern Zetasizer Nano ZS (Malvern Panalytical, Worcestershire, UK). The methodology is based on section 2.3.2 from Foster et al.'s paper on cellulose characterization²¹⁰. DES-treated BC-Pectin and untreated BC-Pectin dispersions with a solid concentration of 0.005 wt.% were prepared by diluting the 1 wt.% dispersions with DI water. To ensure constant ionic activity across all samples measured for zeta potential, a consistent NaCl concentration of 5 mM was maintained.

Small-angle X-ray scattering (SAXS)

Extra Small-angle X-ray scattering (ESAXS), Small-angle X-ray Scattering (SAXS), and Medium-angle X-ray Scattering (MAXS) data of dispersions with a solid concentration of 1

wt.% were collected using a Xenocs Xeuss 3.0 with a wavelength of $1.54 \text{ \AA}^{-1}$ from a $\text{CuK}\alpha$ source. Samples were loaded in a hydrogel stage with wells for different samples. SAXS was collected for 300 s, ESAXS for 600 s, and MAXS for 180 s, covering the q range from 0.004 to $0.1 \text{ \AA}^{-1}$. For background subtraction, DI water and an empty sample well were also measured. The procedures of merging and background subtraction were performed using BioXTAS Raw Software²¹¹.

For power law fitting, the equation $I(q) = Aq^{-n}$ was fit to the data with q ranging from 0.004 to $0.08 \text{ \AA}^{-1}$.

The experimental data was fit using SASview²¹² with a shape-independent model 3.1 containing a two-stage Gauss-Lorentz gel model, taking into account the contribution from the interference between fibers in the network of physical gels²¹³⁻²¹⁵.

$$I(q) = I_G(0) \cdot \exp(-q^2 R_g^2/3) + \frac{I_L(0)}{(1 + ((D + 1)/3)q^2 \xi^2)^{D/2}} + Bkg \quad (3.1)$$

$I(q)$ is the intensity in terms of the scattering vector, q . I_G and I_L are the scaling parameters for the Guinier and Lorentz portions of the equation, respectively, determining the relative contributions of each. R_g is the static correlation length, also referred to as the agglomerate size or entangled region size, which accounts for static accumulations of polymer pinned down by junction points in the polymer network^{213,214}. D is the fractal dimension, which reflects the interactions of fractal objects with their solvent²¹⁶. ξ is the dynamic correlation length, often referred to simply as the correlation length, which describes fluctuations in the position of the polymer chains and is related to the average distance between polymers^{217,218}. Finally, Bkg is the scattering due to the background material. In this study, the background sample was DI water.

Fourier-transfer infrared spectroscopy

Fourier-Transfer Infrared Spectroscopy (FTIR) was performed using a Thermo Scientific Nicolet iS10 FTIR in ATR mode with a diamond crystal. Spectra were obtained with a resolution of 2 cm^{-1} and 64 scans. Scans were performed between 400 and 4000 cm^{-1} . Spectra were normalized by the average absorbance and flattened to ensure absorbance is zero at

3900 and 1800 cm^{-1} . Peak identification was performed using the `scipy.signal.find_peaks` function in python.

Rheology

The rheological tests were conducted using an Anton Paar MCR 301 Rheometer with a double gap measuring system. The amplitude sweep was conducted to determine the linear viscoelastic region (LVR) at 1 Hz in the amplitude range of 0.05 % to 100 %. The frequency sweep was run at an amplitude of 1% from an angular frequency of 0.1 rad/s to 100 rad/s and the viscous response was measured under a controlled shear rate, in the range of 0.001 s^{-1} to 1000 s^{-1} with a measurement time of 60s to 5s varying in logarithmic manner. The creep measurements were performed by applying a step stress (LVR limit stress for each sample) for 600s, then the stress was removed and the strain was measured for 600 additional seconds.

Mechanical testing

Compression tests were performed on an Autograph AGS-X (10kN) load frame from Shimadzu Scientific Instruments, equipped with a 500 N load cell. All test methods were programmed to lower the top compression plate until it came in contact with the sample and registered a force of 0.02 N. For 40% compression tests, each sample was compressed to 40% of its height at a rate of 10% per minute, and then decompressed at a rate of 10% per minute. For cyclic testing, each sample was compressed to 20% of its height at a rate of 10% per minute, and then decompressed at a rate of 10% per minute. This was done 10 times in a row for the full test. For stress-relaxation tests, each sample was compressed to 20% of its height at a rate of 10% per minute, and held there for 20 minutes before being decompressed at a rate of 10% per minute. All mechanical tests were performed 5 times per sample.

3.2. Results

3.2.1 Effects of DES Treatment on Bacterial Cellulose

To promote defibrillation and improve the stability of BC in suspension (Figure 3.1a), we treated the BC with a DES comprised of citric acid and choline chloride, under conditions similar to Xia et al., who used oxalic acid instead of citric acid¹⁴⁷ (see Section 3.1).

In our study, considering that the foams are produced by freeze-drying, we anticipate that the final behavior of the foams will be influenced both by how the fibers are frozen in their solution, and by the DES treatment. We hypothesized that the defibrillation and increased stability of these fibers, achieved through the DES treatment, would improve mechanical properties by creating more homogenous dispersions, and therefore more homogenous foams.

Colloidal and Chemical Properties

To characterize the electrostatic interactions of cellulose fibers prior to lyophilization, zeta potential measurements were conducted on both pure BC in water and DES-treated BC in water. Due to the presence of negatively charged hydroxyl groups on cellulose chains, non-treated BC shows an average absolute zeta potential of 11.7 mV (Figure 3.1b), which is in agreement with prior literature^{219–221}. DES-treated cellulose shows a higher absolute zeta potential of 21.2 mV. Different DES treatments have been reported to either defibrillate BC and increase absolute zeta potential, allowing for more colloiddally stable suspensions^{150,151} and/or to functionalize cellulose¹⁴⁷. In this system, the high aspect ratio and concentration of the BC fibers are in a volume-spanning arrested state (VAS), which creates a percolated network, making the dispersion a colloidal gel (Figure 3.1a)²²². The zeta potential measurements were taken using dilutions of these gels (0.005 wt.%) and do not necessarily provide insight to the colloidal stability of the 1 wt.% gels, but they can give us insight into the electrostatic interactions of the fibers in the colloidal state. If the DES treatment is indeed introducing citric acid functional groups to BC, this nearly doubling of the absolute zeta potential could be explained by the abundance of hydroxyl

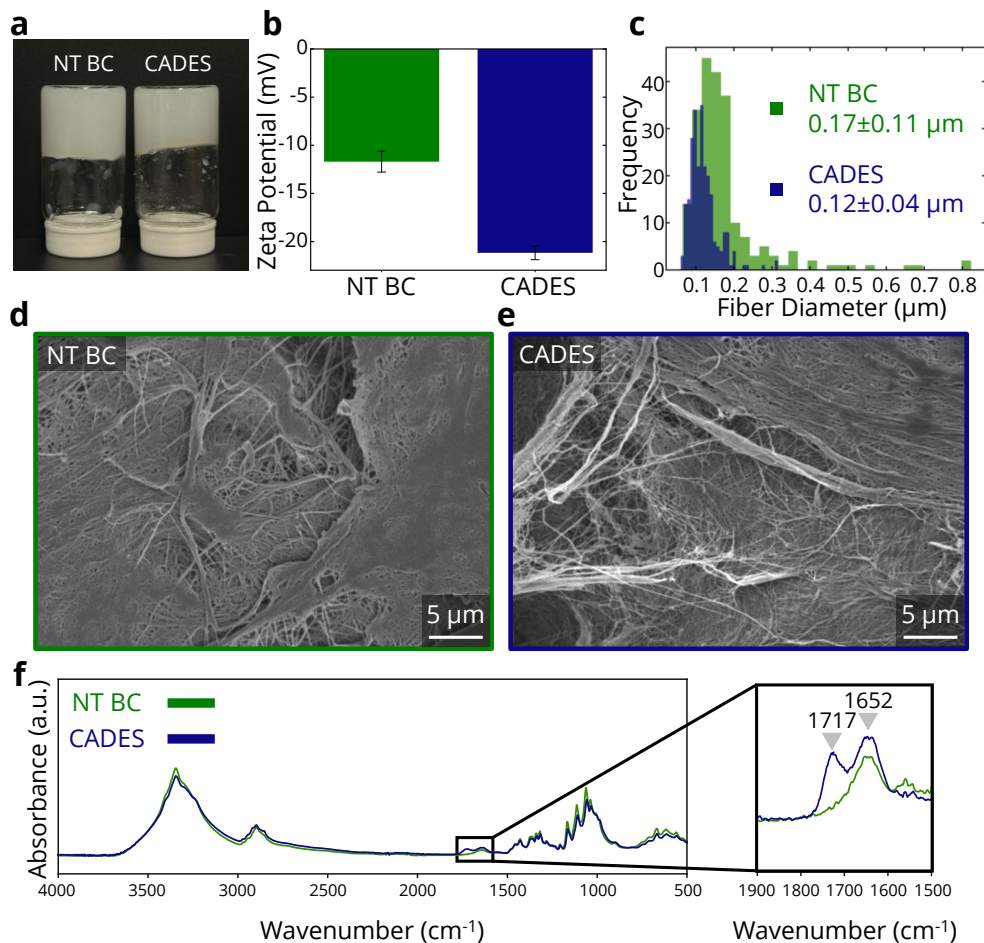


Figure 3.1: **Colloidal and chemical effects of DES treatment on bacterial cellulose.** (a), Non-treated and CADES BC dispersions. (b), Zeta potential of non-treated and CADES BC. (c), Comparison of fiber diameters of non-treated and CADES BC. SEM of non-treated BC (d) and CADES BC (e) films. (f), FTIR spectra of non-treated and CADES BC films.

groups on citric acid. Replacing a cellulose hydroxyl group with a citric acid group increases the amount of hydroxyl groups threefold, thereby increasing the negative charge. However, if the DES treatment is not grafting citric acid functional groups to cellulose, then the increase in absolute zeta potential may be explained by the defibrillation of the cellulose fibers, which would increase the concentration of available hydroxyl groups in a given volume.

Comparing SAXS data for untreated BC and DES-treated BC, we see significantly different behavior induced by the DES treatment (See Table 3.1). The size of the entangled region (R_g) for untreated BC is measured as $\sim 226 \text{ \AA}$ and $\sim 3279 \text{ \AA}$ for DES-treated BC, an order of magnitude difference. This may be explained by the defibrillation of the

Sample	scale	Bkg (cm^{-1})	I_G (cm^{-1})	I_L (cm^{-1})	R_g (Å)	D	ξ (Å)
DES BC	80.55	0.02	320.76	10.75	3278.7	3.24	219.88
BC	0.078	0.00	147.11	10800	225.91	2.90	297.86
20% Pectin	65.09	0.01	247.1	14.16	2607.4	3.13	258.34
40% Pectin	8.37	0.02	202.45	81.19	2149.8	3.19	241.97
60% Pectin	4.40	0.02	152.03	123.21	1760.8	3.18	256.1
80% Pectin	3.85	0.01	175.07	95.81	1955.8	3.10	290.53
100% Pectin	2.86	0.01	2.23	654.9	402.34	2.46	2576.2

Table 3.1: SAXS model fitting parameters.

cellulose fibers due to DES treatment, leading to more overlapping fibers at each point of contact. The DES treatment shows the opposite effect on mesh size (ξ), where the mesh size of the DES-treated BC (220 Å) is smaller than that of the non-treated BC (298 Å). Therefore, the defibrillated DES-treated BC forms a network of entanglements that result in a smaller mesh size, compared to the non-treated BC which forms a significantly looser mesh structure.

To clarify the reasons for the measured differences in zeta potential and SAXS, we conducted SEM imaging (Figure 3.1d-e) to evaluate changes in fibrillation due to the DES treatment. As shown in the SEM images, the DES treated samples consist of finer cellulose fibers which are also more distinct compared to the untreated BC sample surface. Fiber size analysis (Figure 3.1c) reveals an average fiber diameter of $0.17 \pm 0.11 \mu\text{m}$ for the untreated BC sample, while the DES-treated sample had an average fiber diameter of $0.12 \pm 0.04 \mu\text{m}$. The reduced fiber diameter as well as smaller deviation of fiber sizes indicates that the DES treatment conditions enabled the defibrillation of BC.

Next, turning to FTIR spectra (Figure 3.1f) to investigate possible molecular bonding changes due to the DES treatment, we observed a new peak at 1717 cm^{-1} , in the DES-treated BC. This peak indicates the presence of C=O bonds²²³, which are not present in pure cellulose but are found in citric acid. This suggests that cellulose carboxylation occurred through the DES treatment. However, batch-to-batch variation led to inconsistent detection of the carbonyl peak (Figure 3.2), indicating that the functionalization efficiency was inconsistent. Nonetheless, the DES treatment enabled defibrillation in all the studied samples, supporting the higher absolute zeta potential of the DES-treated BC at all cases

in the used reaction conditions.

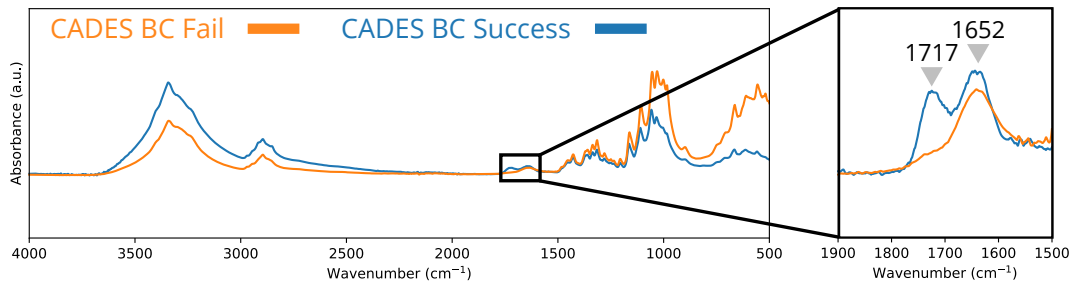


Figure 3.2: **FTIR of failed CADES treatment** FTIR spectra of two different batches of CADES-BC treated under the same conditions.

Morphological and Mechanical Properties of the foams

SEM imaging was used to qualitatively assess morphology and to quantitatively assess pore size of the lyophilized BC foams. The SEM images (Figure 3.4a-b) reveal the porous, cellular structure obtained in both cases. Thicker “cell walls” are observed in the non-treated BC foam compared to the DES-treated case. Higher magnification images (Figure 3.3) show that the “cell walls” in both sets of samples are sheet-like assemblies of BC fibers.

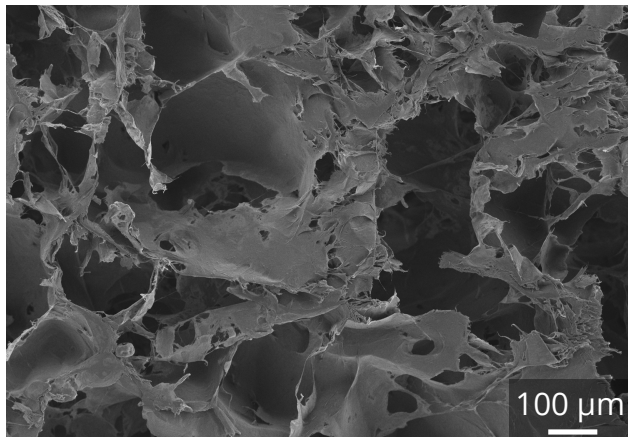


Figure 3.3: **BC Foam Cell** SEM image of CADES-BC foam at 100x.

In the DES-treated foams, the BC fibers within these sheet-like assemblies are more distinct and a higher degree of fibrillation is seen, as shown in Figure 3.1d-e. The pore size histogram (Figure 3.4c) indicates a narrower distribution of pore sizes and a smaller average pore size for DES-treated BC than for non-treated BC. For non-treated BC, the mean pore size was measured at $299 \pm 113 \mu\text{m}$, while the DES-treated BC had a pore size of $117 \pm 73 \mu\text{m}$ (Figure 3.4c). It has been shown that pore size in fibrous foams is influenced by

the aggregation and entanglement of fibers^{224,225}. Munier et al. demonstrated the effects that differences in entanglement and gel networks have on nanocellulosic foams²²⁶. Based on these observations, we hypothesize that, without the DES treatment, the cellulose agglomerates into fiber-rich and water-rich phases, leading to the clusters of fibers and large pore sizes shown in the SEM images. With the DES treatment, the fibers are less agglomerated (in agreement with the zeta potential and SAXS data from the previous section), and therefore create more uniform pore sizes.

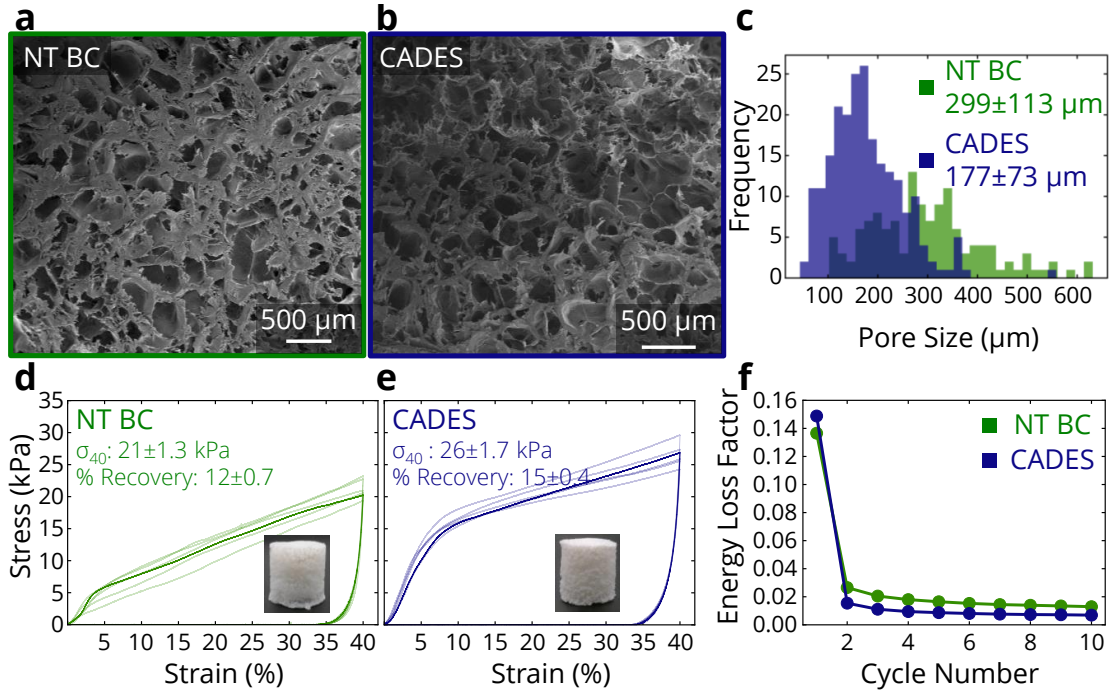


Figure 3.4: Morphological and mechanical effects of DES treatment on BC foams. SEM of non-treated BC (a) and CADES BC (b) foams. (c), Distribution of pore sizes for non-treated and CADES BC foams. Compression tests of non-treated (d) and CADES (e) BC foams. (f), Energy loss factors from cyclic testing of non-treated and CADES BC foams.

To examine how the colloidal properties translate to solid materials, compression tests on lyophilized samples were carried out. In tests where samples were compressed to 40% strain and then unloaded, the DES-treated foams showed a 146% higher loading modulus, and a 25% higher compressive strength at 40% strain (Figure 3.4d-e). The treatment also reduced the variability between samples, as the measured stress-strain responses show smaller differences compared to the non-treated BC. The recoverability of the foam changed negligibly between the non-treated and treated BC.

Furthermore, we conducted cyclic compression tests in which all samples were compressed to 20% strain and unloaded 10 times in succession (representative stress-strain curves shown in Figure 3.5).

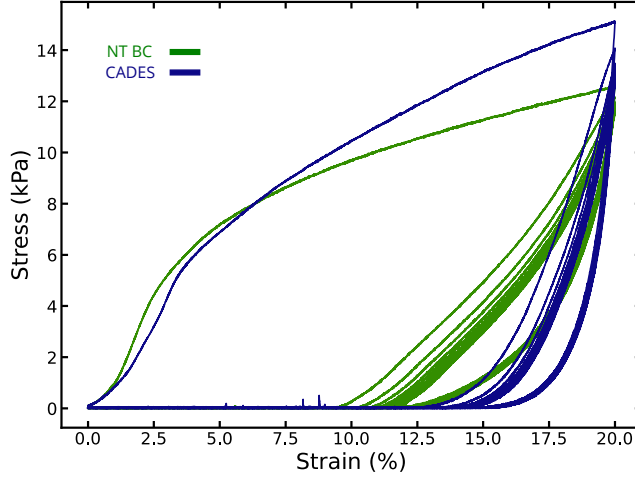


Figure 3.5: **Cyclic tests of NT vs CADES BC** Representative stress strain curves for NT and CADES BC samples under cyclic loading.

The DES-treated samples showed higher compressive strength at 20% strain and more energy absorption in the first compression (1.44 kJ/m³ for CADES-BC compared to 1.26 kJ/m³ for non-treated BC). From the cyclic tests we can calculate the loss coefficient which characterizes a material's ability to dissipate energy in a loading-unloading cycle, and is defined as

$$\eta = \frac{E_d}{2 * \pi * E_s} \quad (3.2)$$

where η is the loss factor, E_d is the energy dissipated during a cycle, and E_s is the energy stored from the first compression^{227–229}. Both foams had a drastic decrease in their energy dissipation ability between the first and second compression cycle (Figure 3.4f), followed by a steady-state response. The CADES-BC exhibited overall lower loss coefficient values (46% lower on average than the non-treated BC, excluding the first cycle). Therefore, the CADES-BC stores relatively more energy than it dissipates compared to non-treated BC.

Overall, the DES treatment of BC leads to a higher absolute zeta potential, fiber defibrillation, and reduced agglomeration. This causes a more homogeneous foam and higher stiffness and strength, as well as the ability to store more energy during cyclic compression than non-treated BC.

3.2.2 Effects of Pectin in BC foams

After understanding the properties of pure BC foams with and without DES treatment, we aimed to expand the attainable properties, specifically to improve the recoverability of the foams. A way to achieve a modular mechanical property change is to create a binary network with another polymer. Given the higher colloidal stability, defibrillation, strength and stiffness of the produced foams obtained after DES treatment, we chose that as the primary polymer network in our composite system. Pectin’s molecular structure, as in nature, allows for a strong interacting network with the strong and stiff cellulose fibers, creating a hydrogen bond-dominated network where the softer pectin chains enable more extended deformation under loading.

As described in the Methods section (3.1), we introduced water-dissolved pectin at a 1 wt.% concentration into the 1 wt.% CADES-BC gel in the required ratios to achieve a dry material of the desired concentrations of pectin and BC. The produced colloids were all homogenous (Figure 3.6a inset) as expected, with no phase separation or precipitation noted. The binary network colloids were subsequently lyophilized to produce composite foams.

Colloidal Properties

First, we studied the electrostatic stability of colloids through zeta potential measurements (Figure 3.6a). Our data show that a pure pectin solution has a zeta potential of about -20 mV, similar to the DES-treated BC (-21 mV) and higher (in absolute value) than the non-treated BC (-12 mV). When pectin is introduced to the BC dispersion, we see that the absolute zeta potential increases higher than either the BC or the pectin. This may indicate that the pectin-BC networks have higher surface charge densities than either component alone, bringing about a synergistic effect between the two components. Interestingly, the pectin used in this study was acidic, and cellulose in acidic conditions typically has a zeta potential closer to zero²²¹. Therefore the interactions with the pectin seem to cause the opposite behavior than expected.

To better understand the entanglements in the colloidal system, SAXS was performed

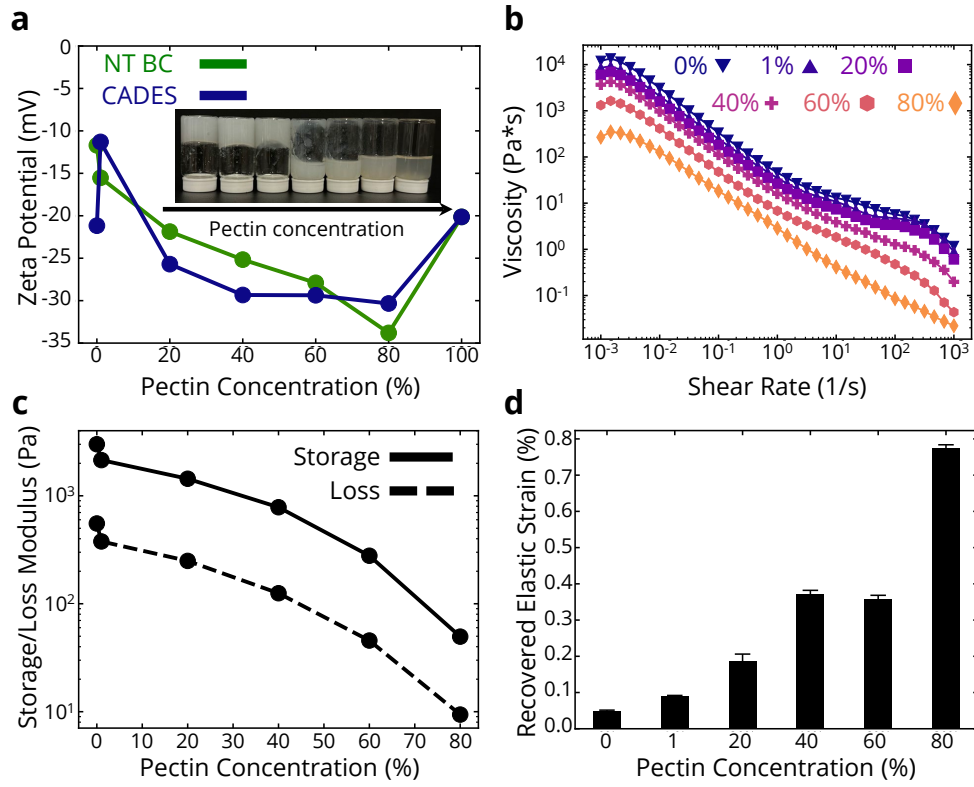


Figure 3.6: **Effects of Adding Pectin on Colloidal Properties.** (a), Zeta potential measurements of non-treated and CADES BC dispersions with varying amounts of pectin added. Viscosity measurements (b) and storage/loss moduli at ~ 5 rad/s (c) of CADES BC/pectin dispersions (d), Recovered elastic strain percentage of CADES BC/pectin dispersions.

on the samples. Using a power law fit, we can get an idea of the type of network formed by the BC-pectin gels (Figure 3.7a and Table 3.2). Note that the power is approximately 2 for all BC/pectin composites, indicating the presence of an entangled system²³⁰.

Pectin %	Power	Scale	R ²
0.0	2.01	0.003	0.99
20.0	2.055	0.002	0.99
40.0	2.05	0.002	0.99
60.0	2.04	0.001	0.99
80.0	2.00	0.001	0.98
100.0	2.87	0.000	1.00

Table 3.2: Power law fitting parameters of SAXS data

By using a combination of the Guinier and Lorentz models (see 3.1), we can investigate the agglomerate size and correlation lengths of the colloidal systems. In equation 3.1, R_g represents the agglomerate size, or the size of an entangled region pinned down by

junctions^{213,214}. ξ is associated with the thermodynamic fluctuations of polymer chains²¹⁴ and is related to the average distance between polymers due to the screening of excluded volume interactions^{217,218}. In Figure 3.7b and c, we see that the agglomerate size generally decreases with pectin concentration, while the correlation length generally increases with pectin concentration. This suggests that pectin molecules shield cellulose chains from each other, reducing the amount and size of cellulose agglomerates and increasing the size of the mesh in the cellulose-pectin matrix. Figure 3.7d shows that the reptation tube diameter increases with pectin concentration. The reptation tube indicates the path along which a polymer chain's center of mass moves due to Brownian motion. Recognizing limitations of using reptation theory in semiflexible polymers²³¹, such as BC and pectin, we can still get useful insights. We first calculated the tube diameter using the relationship $d \propto \xi^2 R_g^{-1}$ ²³¹. The aforementioned shielding due to pectin molecules may also explain the increase in reptation tube diameter, as this shielding would increase the excluded volume of the cellulose polymers, giving more space for each polymer to move. Additionally, Figure 3.7e shows that the storage modulus, taken at the LVR limit from the amplitude sweeps, decreases with reptation tube diameter. With the motion of polymer chains confined to a tube-like region, a larger tube diameter would result in increased chain mobility, leading to a decrease in storage modulus.

Rheology was used to further analyze the colloidal behavior of the BC-pectin gels. The flow curves in Figure 3.6b show that increasing pectin concentration reduces the viscosity of the gel at all shear rates. We see smaller effects for lower concentrations of pectin, where the reduction in viscosity at a shear rate of 1 s^{-1} is $\sim 43\%$ when adding 20 wt.% pectin. At higher pectin concentrations, the viscosity reductions are greater, such as going from 60 wt.% to 80 wt.% causing a $\sim 58\%$ reduction in viscosity at 1 s^{-1} . A reduction in both the storage and loss moduli is observed as pectin concentration is increased (Figure 3.6c). Again, we see smaller effects of pectin at lower concentrations, where the reduction in storage modulus is $\sim 53\%$ when adding 1 wt.% pectin. At higher pectin concentrations, the effects are increased, showing a $\sim 83\%$ reduction in storage modulus when going from 60 to 80 wt.% pectin. Amplitude sweep curves for the dispersions are shown in Figure 3.8.

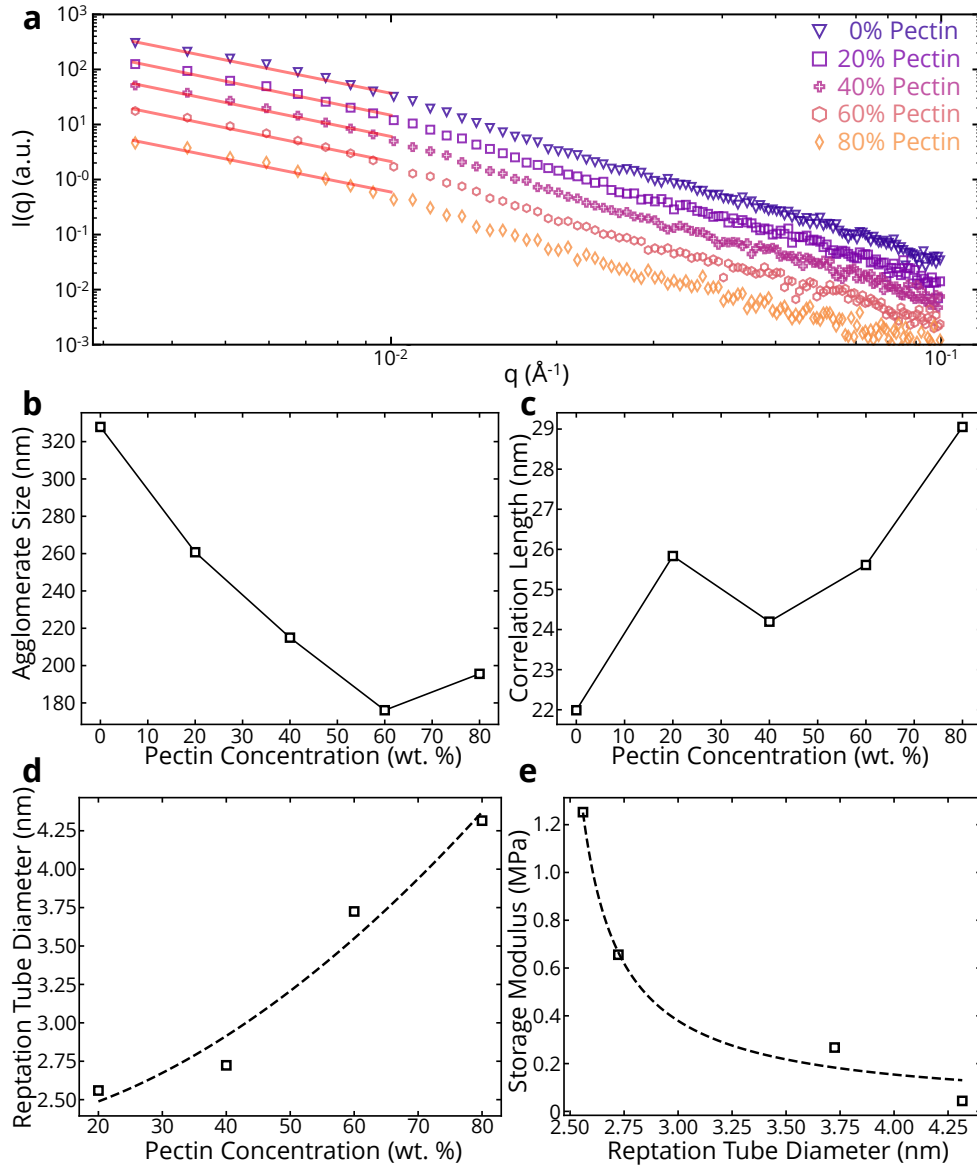


Figure 3.7: **Effects of Adding Pectin on Network Properties via SAXS.** (a), SAXS data for CADES BC/pectin composites with a power-law fit in the low- q region. (b), Agglomerate size, (c), correlation length, (d), and reptation tube diameter of CADES BC/pectin composites. (e), Effect of reptation tube diameter on storage modulus of the colloids.

These curves show clear viscoelastic behavior, and the storage modulus is higher than the loss modulus for all composites that have some amount of BC in them, until a crossover strain at which point the loss modulus dominates.

The gels have a non-brittle fracture, as indicated by the crossover points being in the negative slope portion of the curves. This is consistent with observations of the behaviors of the gels; they have a clear but weak network structure, and the relatively low viscos-

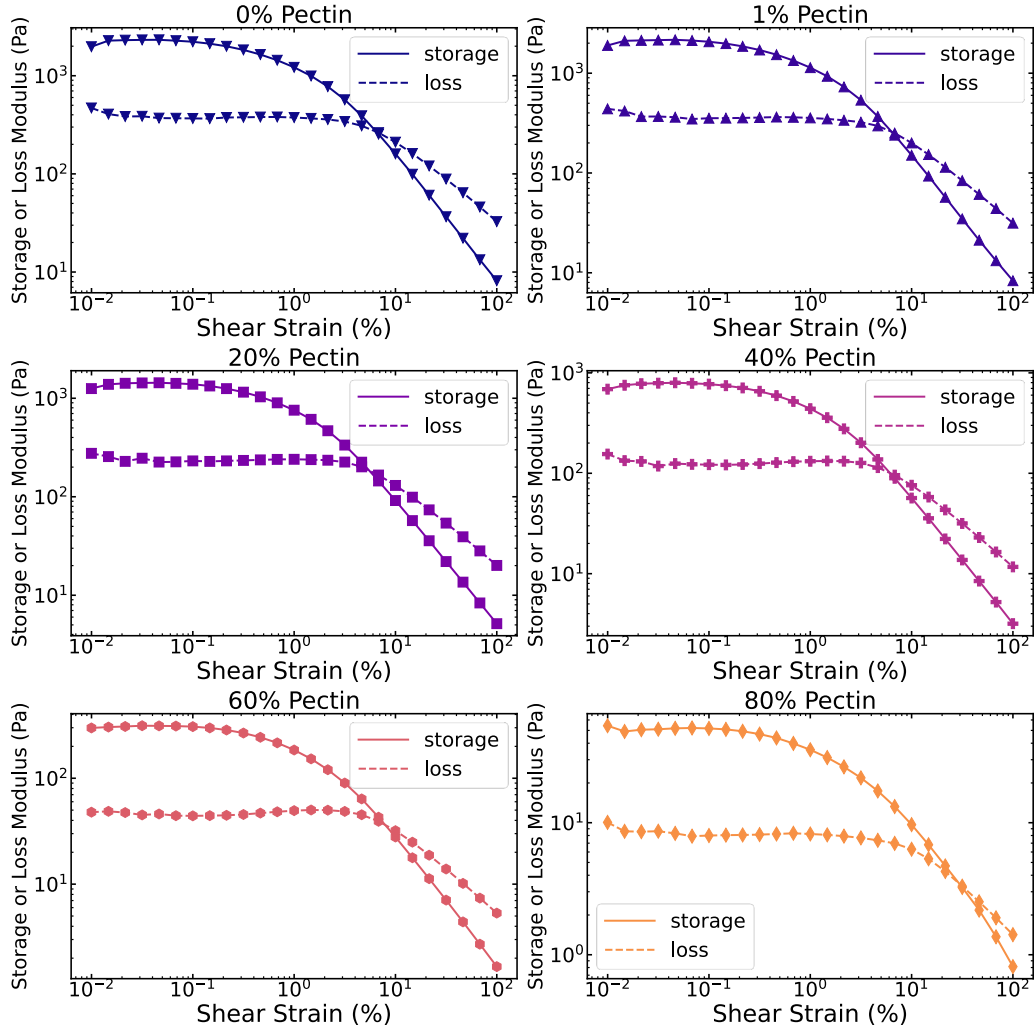


Figure 3.8: **Amplitude Sweeps of CADES-BC/Pectin Colloids.** Storage and loss modulus curves from the amplitude sweep tests on the CADES-BC/pectin colloids.

ity allows for flow under gravity at higher pectin concentrations, as shown in the inset of Figure 3.6a. Combined, as expected, we see that introducing pectin in the stiff cellulose network leads to an electrostatically stable binary network with reduced rigidity and stiffness. We note that in this study, we used deionized water as our dispersion medium. A means to improve the network strength would be to provide cations in the system which would promote ionic crosslinking in the pectin chains^{232,233}.

To analyze the time-dependent behavior of our binary networks, we conducted creep tests, as shown in Figure 3.9 and 3.6d. Constant stress was applied for 10 minutes and released for another 10 minutes, allowing us to measure the recoverable strain of the gel network.

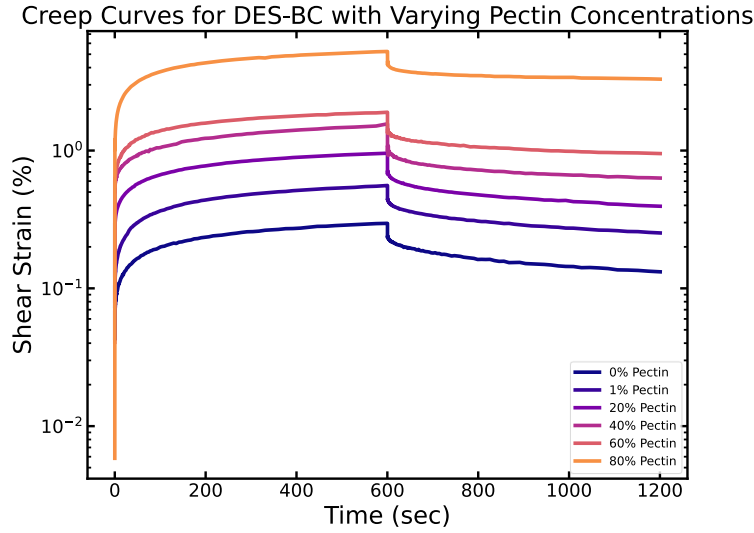


Figure 3.9: **Creep tests of CADES BC/Pectin Composites** Representative curves for CADES BC samples under rheological creep testing.

To characterize the ability for the foams to elastically recover after the release of stress, the following equation was used to first model the non-elastic recovery

$$\epsilon_{rvis}(t) = \epsilon_r [e^{-(t/\eta_r)^{\beta_r}}] + \epsilon_f \quad (3.3)$$

where ϵ_{rvis} is the total strain in the non-elastic recovery region, ϵ_r is the viscoelastic strain, η_r is the scale parameter, β_r is the shape parameter, and ϵ_f is the remaining viscous strain. To get the recovered elastic strain, the equation below was used

$$\epsilon_{recov} = \epsilon_{rvis} + \epsilon_e \quad (3.4)$$

where ϵ_{recov} is the total strain from the point of unloading, ϵ_{rvis} is the non-elastic recovered strain, and ϵ_e is the purely elastic strain, which is recovered instantly upon unloading^{234–237}.

Increasing the pectin content increased the recovered elastic strain by $\sim 87\%$ at 1 wt.% pectin and over 16x at 80 wt.% pectin. The recovered viscoelastic strain, ϵ_r , also increased with pectin concentration (SI Figure 3.10). The increasing recoverable elastic and viscoelastic strain in the presence of pectin could be due to the improved elastic fiber mobility in the network, afforded by the presence of pectin macromolecules.

Overall, the above analyses of the BC-pectin colloids show that the binary network

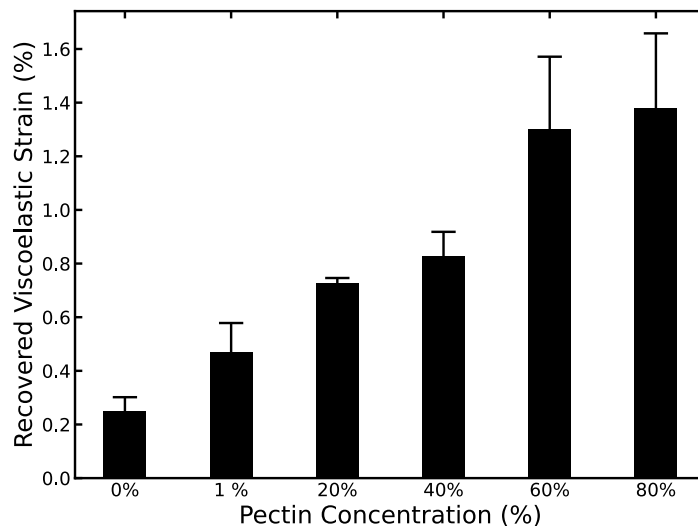


Figure 3.10: **Recovered Viscoelastic Strain.** Viscoelastic strain recovered during creep-recovery tests of CADES-BC/Pectin colloids.

is more stable in water than either of the pure component gels (as shown by the more negative zeta potentials of the composite gels), and that the presence of pectin affords an improvement in the network’s recoverability at the expense of gel stiffness. The presence of pectin appears to create a weaker network, but due to electrostatic repulsion there may be less fiber entanglements, allowing for fiber rearrangements during loading. Next, we will examine how these colloidal properties translate to solid properties of the lyophilized foams.

Mechanical and Morphological Properties

The SEM images in Figure 3.13a and b reveal the micromorphological changes upon incorporation of pectin in the BC foams. At 0 wt.% pectin (pure CADES-BC sample shown in Figure 3.4a-b), we saw a relatively open cell structure (as discussed in Section 3.2.2), where BC fibers assemble to form thin sheet-like “cell walls”. In the 20 wt.% pectin foam (Figure 3.13a) we still see thin sheets serving as “cell walls” in a mostly open cell structure, but, in this case, the BC fibers are clearly immersed in a continuous pectin matrix switching from a fibrillar to a fiber-in-matrix morphology. In plant cell walls, cellulose fibrils are held within a matrix consisting of up to 35% pectin, which can affect the mechanical behavior of the cellulose network²³⁸. It therefore is reasonable that we see a fiber-in-matrix morphology in our samples as well. When increasing pectin amount to 80

wt.% (Figure 3.13b), the pectin-rich matrix becomes more prominent, the cellulose fibers are less distinct, and there are fewer smaller pores throughout the foam. In the pore size distribution comparison of these two images 3.11, we see an almost bimodal distribution of pore sizes for the 80 wt.% pectin composite. The regular pore sizes are in the 200-300 μm range, while the “tears” in the cell walls correspond to the node around 50 μm . For the 20 wt.% pectin composite, we see a higher number of smaller pores, although based on the SEM image, we can determine that these are mostly holes in the BC/pectin matrix. The change in “cell wall” morphology along with the changes in pore structure, suggest that the impact of introducing pectin to BC foams on the mechanical properties will be significant.

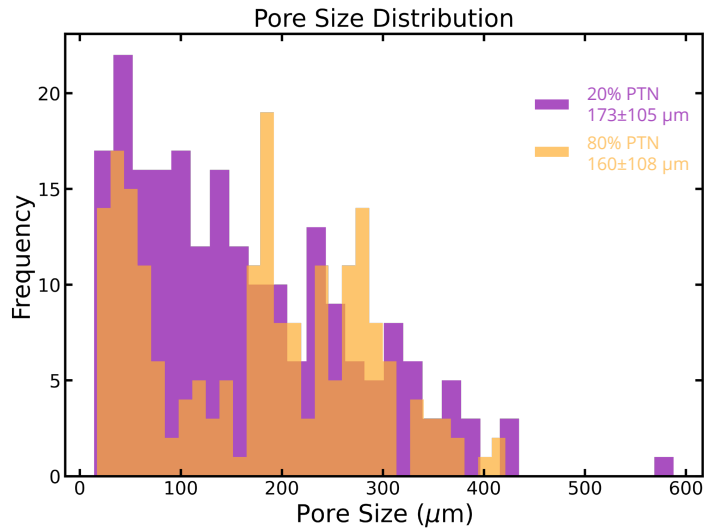


Figure 3.11: **Pore size distributions for CADES-PTN Composites** Histograms of the pore size distributions of CADES-PTN composites at 20%/80% and 80%/20% concentrations.

Indeed, compression tests show a wide property space for BC-pectin foams depending on pectin concentration. Using the 40% compression testing (Figure 3.14a and Figure 3.12), we see that compressive strength values at 40% strain increased by approximately 26% and the loading modulus increased by about 10% at 20 wt.% pectin concentrations.

Thus, a synergistic effect was observed for the strength and modulus of the binary network foams with the lowest tested pectin content. However, strength decreased after 20 wt.%, dropping down to 7.6 kPa at 100 wt.% pectin (an approximately 72% decrease compared to pure CADES-BC). On the other hand, the recovery of the foams increased sig-

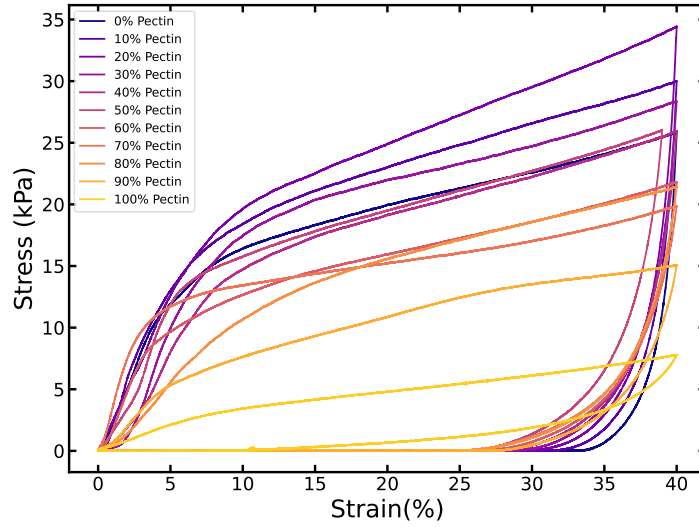


Figure 3.12: **Pectin sweep stress-strain curves** Representative stress-strain curves of CADES BC/pectin composites.

nificantly with pectin concentration (Figure 3.14b). At 20 wt.% pectin, where compressive strength was the highest, the foams exhibited 26% recovery, compared to 15% recovery for pure CADES-BC (a $\sim 73\%$ increase). The recovery percentage increases steadily, getting to almost 40% recovery at 90 wt.% pectin. Pure pectin foams show a recovery of about 68%, indicating the presence of even 10 wt.% BC significantly decreases the recoverability of BC-pectin composite foams. The increasing recoverability with increasing pectin concentration matches with what we saw in the rheological creep tests (Section 3.2.2), where the increase in pectin increased the recovered strain of the gels. The data suggest that the presence of pectin adds elastic energy absorption pathways in both the gel and solid composites.

Overall, the lowest amount of pectin probed in our experiments enabled a synergistic improvement in the compressive strength and modulus of the foam, exceeding the properties of pure CADES-BC, while also enabling an improvement of the recoverability. The addition of pectin reduces the amount of irrecoverable strain in the foams, and it creates a fiber-in-matrix morphology that improves the load transfer between BC fibers.

To further examine the effects of pectin on BC foams, we conducted dynamic mechanical tests. Stress relaxation tests (Figure 3.14c) show an increase in the stress decay, which indicates how much energy is dissipated while the compressive strain is held constant.

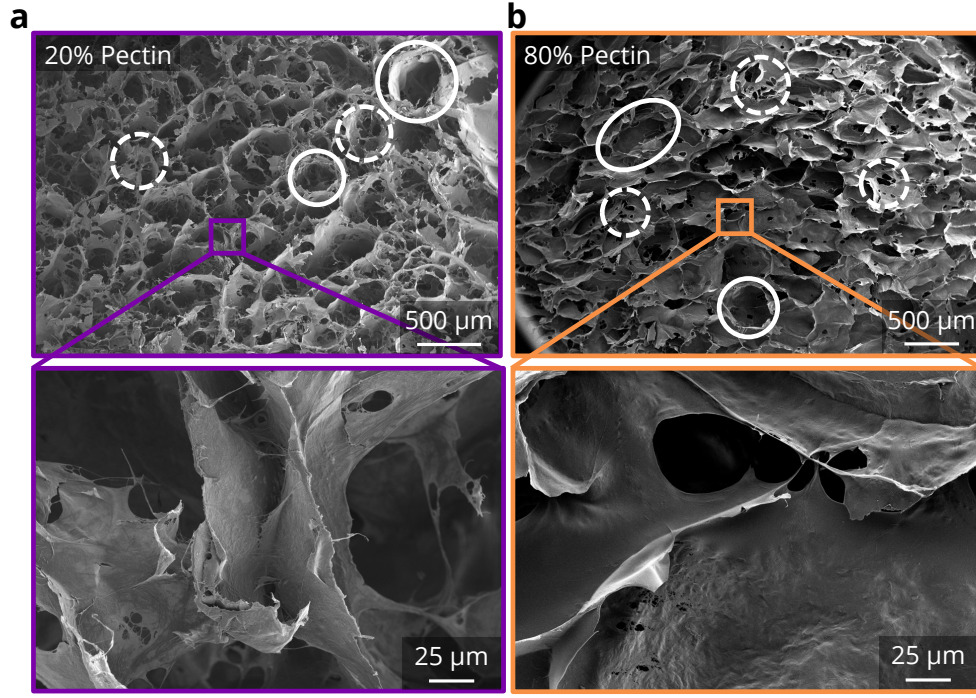


Figure 3.13: **Effects of Adding Pectin on Morphological Properties.** SEM images of a CADES BC foam with (a) 20% pectin and (b) 80% pectin at 30x (top row) and 500x (bottom row) magnifications. Solid circles indicate cells in the foams, dashed circles indicate holes in the matrix.

Stress decay is calculated as the ratio of the stress lost during the relaxation period to the stress at the start of the relaxation period, as shown below:

$$\text{stress decay} = \frac{\sigma_{max} - \sigma_{end}}{\sigma_{max}} \quad (3.5)$$

where σ_{max} is the maximum stress, or the stress at the start of the relaxation period, and σ_{end} is the stress at the end of the relaxation period.

An increase in stress decay shows an increase in the ability for the matrix to accommodate the stress being applied to it, demonstrating an increase in the chain mobility of in the binary system. Pure pectin samples have a significantly higher stress decay, and therefore higher chain mobility, than pure BC foams. As pectin concentration increases, the overall chain mobility in the foam also increases.

This conclusion is further supported by cyclic testing, as shown in Fig 3.14d. All materials show a non-linear dependence of loss coefficient, with the first compression causing the greatest energy loss and subsequent compressions showing very small drops in energy

EPS, PE, and PU foams with similar apparent density. In Figure 3.14e we report the measured unloading moduli versus density for all tested materials (representative stress-strain curves at Figure 3.17). The BC/pectin foams fall within and exceed the range of moduli of the synthetic foams. Our foams have unloading moduli ranging from 0.2 to 2.6 MPa at densities of 0.012 ± 0.001 g/cm³ on average. Synthetic commercial foams have unloading moduli ranging from 0.3 to 0.8 MPa at densities ranging from 0.007 g/cm³ to 0.030 g/cm³.

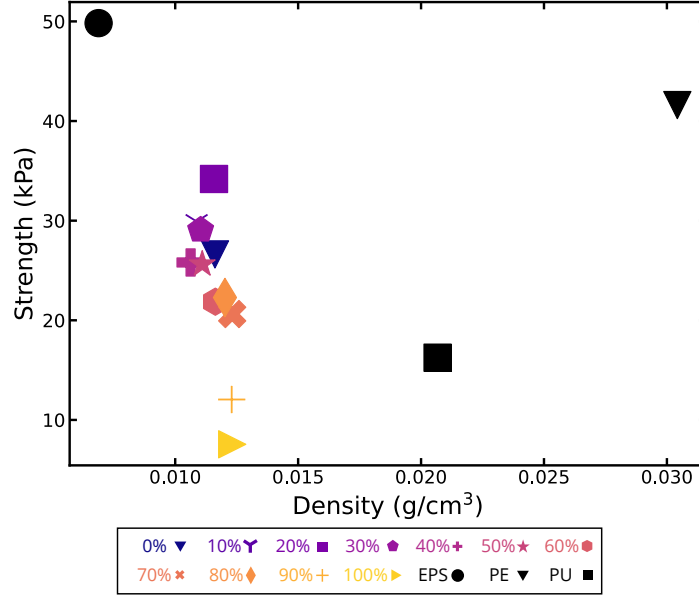


Figure 3.15: **Strength at 40% strain vs density** A comparison of the compressive strength at 40% strain vs density of the BC/pectin foams and the synthetic polymer foams.

When comparing strength, we similarly see that the BC/pectin foams are on the same order of magnitude in terms of strength (Fig 3.15). Our foams have strengths ranging from 7.6 kPa to 34.2 kPa, while the synthetic foams range from 16.3 kPa to 49.8 kPa.

However, if we compare the extent of recovery of the foams after unloading, we see that the recoverability of the synthetic polymer foams far exceeds that of our BC/pectin foams, showing the significant gap in mechanical properties that still exist (Fig 3.16). Our foams have recoverabilities ranging from 15% to 68%, while commercial foams have recoverabilities ranging from 54% to 92%.

One stark difference between the tested synthetic foams and our biopolymer foams, besides the chemical structures, is that the synthetic foams are all closed-cell foam structures. Our biopolymer foams are all open-celled foam structures, which causes a significant

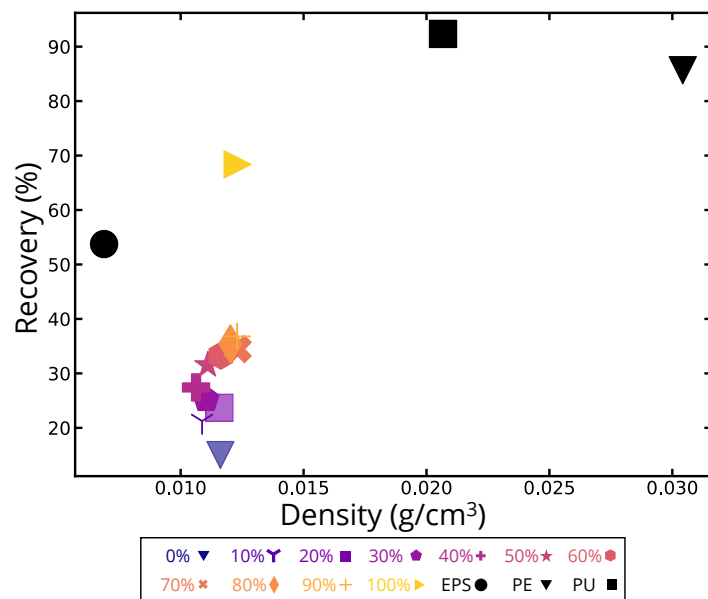


Figure 3.16: **Recovery vs density** A comparison of the recovery vs density of the BC/pectin foams and the synthetic polymer foams.

change in the mechanical behavior of the foams. With the current preparation method that applies lyophilization on the colloids to produce the foams, it is not possible to achieve a closed-cell structure with which to compare to the synthetic foams.

3.3. Conclusion

In this Chapter, we investigated the effects of DES treatment on BC, and the incorporation of pectin to enhance the properties of BC foams. The DES treatment with citric acid and choline chloride successfully promoted defibrillation, leading to finer and more stable cellulose fibers, as evidenced by SEM and zeta potential measurements. This treatment also introduced functional groups, as indicated by FTIR spectra, though with inconsistent efficiency. The resulting DES-treated BC foams exhibited improved mechanical properties, including higher loading modulus and compressive strength, compared to non-treated BC foams. However, upon cyclic compressive loading the CADES-BC foams had slightly lower loss coefficient values, potentially indicating a lesser ability to dampen and disperse energy.

Incorporating pectin into the CADES-BC foams created a binary network that further modified the mechanical and colloidal properties. Pectin, which is acidic in solution, increased the absolute zeta potential of BC/pectin composite dispersions, which is the op-

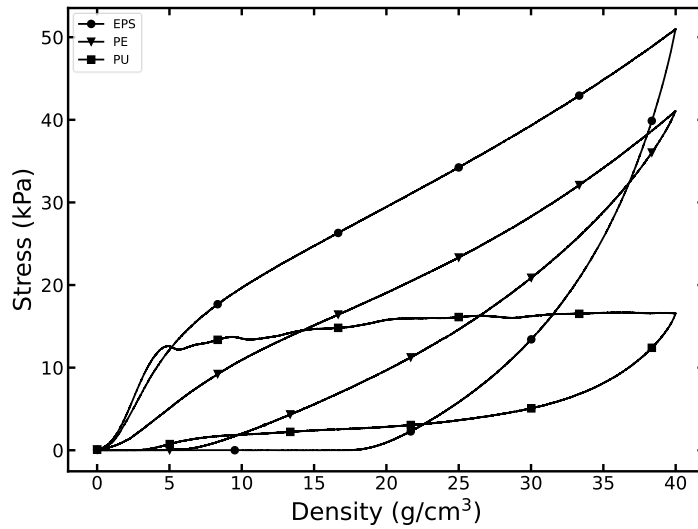


Figure 3.17: **Commercial polymer stress-strain curves** Representative stress-strain curves of expanded polystyrene, polyethylene, and polyurethane foams.

posite of what is usually expected for cellulose in acidic environments. Additionally, the absolute zeta potential of the composites was higher than that of either of the constituent components, suggesting a synergistic interplay in the binary network. The incorporation of pectin also reduced the viscosity and the storage/loss moduli, with higher pectin concentrations leading to higher decreases. Rheological creep tests showed that an increase in pectin increased the colloid’s ability to recover strain after a constant stress was removed, demonstrating the pectin’s ability to improve the fiber mobility in the network.

Morphological analysis revealed a transition from a fibrillar to a fiber-in-matrix structure upon introduction of pectin in the BC foams, improving flexibility and recoverability of the foams. Strength at 40% strain was also found to increase up to 20 wt.% pectin but decreased at higher concentrations. Recovery of the foams consistently increased as pectin concentration increased, and the ability of the foams to dissipate energy under constant load increased at higher pectin concentrations as well.

Overall, our findings indicate that DES treatment and pectin incorporation are effective strategies for tuning the properties of BC foams. The treated and composite foams exhibited promising mechanical performance, with potential applications in areas requiring lightweight, high-strength, and energy-absorbing materials. We also showed that these biomatter-based foams can be competitive with petroleum-derived foams, and therefore

can provide a path forward for sustainability in packaging materials. Future work includes optimizing the functionalization process and exploring the potential for closed-cell foam structures to further enhance the mechanical properties.

4. Algal Foams for Acoustic Absorption

This chapter is based on the following submitted work:

Hareesh Iyer, Mohammad Tabatabaei Manesh, Meghan Dillon, Ryan Kim, David Kim, Tomás Méndez Echenagucia, Eleftheria Roumeli. Biodegradable, self-extinguishing foams made from *Ulva* for broadband acoustic absorption. Under Review.

4.1. Materials and Methods

4.1.1 Material Preparation

One species of *Ulva* was collected on June 5th, 2024 from Thorndyke Bay, Washington, with permission, on a section of beach owned by Baywater Shellfish Company. The species of this wild *Ulva* was not known, so it is henceforth referred to as *Ulva sp.* or *U. sp.* *Ulva lactuca*, hereinafter *U. lactuca*, was purchased from Maine Coast Sea Vegetables as a dry, flaky powder. Note that the supplier’s website states that they get their *Ulva* from cold, North Atlantic waters, while *U. lactuca* typically is found in tropical waters. This is likely due to taxonomic issues with *Ulva*, as it was recently determined that North Atlantic *U. lactuca* is actually *U. fenestrata*²³⁹. Still, this material will be referred to as *U. lactuca* for the rest of this paper. *Ulva ohnoi*, hereinafter *U. ohnoi*, also in the form of dry flakes was kindly supplied by Pacific Northwest National Laboratory.

To process the *U. sp.*, we first washed it with tap water to remove sand, crabs, and other detritus. It was then dried in a Presto 06300 Dehydro Electric Food Dehydrator at 35 °C for 24 hours before being broken up into smaller flakes. All *Ulva* powders were then powderized using a SpeedMixer DAC 330-100 PRO from FlackTek in Landrum, SC, USA. An 8 ml stainless steel SpeedMilling jar with three grinding rings was used with 2 grams

of material at a time, run at 2500 rpm for 3 minutes total with 30 seconds of cool-down time in the middle.

Ball-milled powder was then mixed into deionized (DI) water at concentrations of 5, 7.5, and 10 wt.%. The slurries were then sonicated using a Fisher Scientific Model 505 probe sonicator from Thermo Fisher in Waltham, MA, USA. A 0.5 inch diameter probe was used for sonication at 80% amplitude for 10 minutes with 3 minutes on / 3 minutes off cycles. The beakers holding the slurries were kept in an ice bath during sonication to prevent overheating of both the samples and probe.

To make acoustic foam samples, the slurries were poured into 250 ml FlackTek SC 250 cups up to a height of approximately 25 mm. To make mechanical test samples, the slurries were poured into custom cylindrical 3D-printed molds with nominal inner dimensions of 15 mm in height and 15 mm in diameter. Slurries were then kept in a refrigerator for at least 12 hours before being moved to a standard freezer for at least 12 hours. Finally, the samples were lyophilized using a Labconco Corp. (Kansas City, MO, USA) bench top freeze dryer, catalog number 700201000. Samples were kept in the freeze dryer with a collector temperature lower than -40 °C and a chamber pressure less than 0.100 mbar until the samples were fully dry, which usually took about 2 days for the mechanical test samples and one week for the acoustic samples. Foam samples with different porosities are henceforth referred to by the concentration of algae that was in the slurry prior to freeze drying.

4.1.2 Optical Microscopy

Optical microscopy was done using a Panthera TEC-BF microscope (Motic, Hong Kong). For *U. lactuca* and *U. ohnoi* powders, water was placed on a glass slide and a small amount of algae powder was sprinkled over the water. A glass cover slip was used to cover the material. For *U. sp* blades, the entire tissue was laid on the microscope table with a glass slide placed on top to flatten the viewing area.

4.1.3 Scanning Electron Microscopy (SEM)

SEM was done using a Phenom ProX SEM (NanoScience Instruments, Phoenix, AZ, USA) on samples that were sputter coated with gold for 30 seconds. Pore sizes were measured using ImageJ²⁴⁰.

4.1.4 Fourier Transform Infrared spectroscopy (FTIR)

FTIR was done using a Perkin-Elmer (Waltham, MA, USA) Frontier FTIR in ATR mode. 64 scans were run from 400 to 4000 cm^{-1} with a resolution of 0.5 cm^{-1} . Curves were flattened to keep absorbance equal to zero between 1800 and 3900 cm^{-1} and normalized by the mean intensity.

4.1.5 Mechanical Testing

Mechanical testing was done using an Autograph AGS-X from Shimadzu Scientific Instruments (Columbia, MD, USA). All tests were done using compression plates and a 500 N load cell. All test methods were programmed to lower the top compression plate until it came in contact with the sample and registered a force of 0.02 N. For 40% compression tests, each sample was compressed to 40% strain at a rate of 10% per minute, and then decompressed at a rate of 10% per minute. For strain rate comparisons, each sample was compressed, then decompressed to 20% strain at strain rates of either 0.1% per minute, 1% per minute, or 10% per minute. For cyclic testing, each sample was compressed to 3% strain at a rate of 10% per minute, and then decompressed at a rate of 10% per minute. This was done 10 times in a row for the full test. For cyclic tests with varying strain, samples were compressed to 2%, 5%, 10%, and 20% strains at a rate of 10% per minute, in succession, with unloading steps in-between at the same strain rate.

4.1.6 Statistical comparisons

Comparisons of mechanical test were done using a Welch's t-test, implemented using the `ttest_ind` package from `scipy.stats`, with `equal_var` set to `False`. Comparisons of size distributions were done using a Mann-Whitney U-test to compare the medians of the datasets,

implemented using the mannwhitneyu package from scipy.stats.

4.1.7 X-ray Computed Tomography (Micro-CT)

Micro-CT was performed on foam samples that were prepared in the same way as the acoustic samples, using an X5000 Industrial CT Scanning System from North Star Imaging (NSI, Rogers, Minnesota, USA). These samples were not trimmed to 40 mm diameters prior to scanning. All samples were imaged at the same position relative to the source and detector with a source current of 700 mA, a voltage of 60 kV, and a focal spot size of 42 μm . The detector had a pixel pitch of 200 x 200 μm , a gain of 0.25 pF, no binning, a framerate of 2 fps, and a region of interest (ROI) of 2048x2048+0+0. A defect map was used to account for defective pixels in the scans. Each scan had a geometric unsharpness of 0.995 pixels (0.199 mm). 2900 projections were produced from each scan, with a range of rotation of 360 degrees in continuous scan mode. For reconstruction, a scan of a standard geometry object provided by NSI was performed as reference at the same position as all other samples. Slices were exported in only the Y-direction via the EFX-CT reconstruction software from NSI. Analysis of the 3D images constructed from the slices was done separately, as explained in the following sections.

Segmentation

Micro-CT data was analyzed using Dragonfly 3D World²⁴¹. Datasets were trimmed to a 40 mm diameter cylinder to match the size of the acoustic samples. These 3D renderings of the samples were segmented into pore and material ROIs by adjusting the lower limit of the intensity histogram and exporting the resulting ROI (material), its inverse (pores), and a sum of the two (full sample). Then, the porosity was checked against the calculated porosity for that sample by dividing the voxel count of the pore ROI by the voxel count of the full sample ROI. The above steps were repeated until the porosity measured in Dragonfly matched the porosity measured from pycnometry and apparent density (see Section 4.1.14). For *U. sp*, the process required extra steps due to the high intensity of the inorganic particles compared to that of the algae. To account for this, the volume percentage of the highest intensity voxels was measured using the intensity histogram to

segment out the inorganic particles. The volume percentage of the algae was then calculated by subtracting the volume percentage of inorganics and the porosity from 100. An ROI was then created for the algae in the same way that an ROI was created for the pores for non-*U. sp* species. Once both the algae and inorganic ROIs were created and matching the measured/calculated volume percentages, a pore ROI was created by subtracting the algae ROI from the combined algae and pore ROI. Once a pore ROI was created for a sample, a dense graph was created from the pore ROI and tortuosity was calculated from the graph in the y axis direction. This calibration procedure ensured an optimal match between experimental and analytical results, enabling accurate material characterization for subsequent numerical simulations.

Tortuosity

A box shape object was created at both the +Z and -Z ends of the ROI, extending up to 3 mm towards the center. A graph was then created for each ROI using the “Create Dense Graph” function from the 3D Modeling menu. The tortuosity was then calculated using the “Graph Tortuosity Analysis” function with the +Z box as the input and the -Z box as the output. Mean tortuosity was used as the representative tortuosity value of each sample.

Micro-CT Pore Size Analysis

Each ROI, which was 40 mm in diameter, was divided into smaller cylindrical ROIs of 30 mm for *U. lactuca*, *U. ohnoi*, and *U. sp* at 10 wt.%, 15 mm for *U. sp* at 7.5 wt.%, and 10 mm at 5 wt.%. This was done to reduce the computational demands for running the calculations. Graphs and multi-ROIs representing the pore networks in each ROI were created using the OpenPNM interface within Dragonfly 3D. Phase Index was set to 1, Sigma to 0.4, R max to 4, and Edge tolerance to 0.5 %. The options to trim isolated pores and label faces were not selected. Equivalent diameters, minimum Feret lengths, and maximum Feret lengths for the pores were then calculated for each multi-ROI created by OpenPNM.

4.1.8 Rheology

Rheology was done on an MCR 102e from Anton Paar (Graz, Austria). Viscosity tests were performed on slurries with 5, 7.5, and 10 wt.% concentrations of ball-milled algae powder in deionized water.

For the *U. ohnoi* 7.5% and 10% samples and *U. lactuca* 10% samples amplitude sweep testing, samples were prepared by pipetting the slurries onto wax paper within individual petri dishes immediately after ultrasonication and were allowed to sit in a refrigerator overnight. Samples were loaded onto the rheometer plate by lifting the wax paper along with the gel out of the petri dish, placing the gel-side down onto the sandpaper, and gently peeling the wax paper away from the gelled *Ulva*. For *U. sp* samples, 5% *U. ohnoi* samples, and *U. lactuca* 5% and 7.5% samples, slurries were pipetted directly onto the rheometer plate after sitting in a refrigerator overnight. All three species of *Ulva* were tested with the same parameters for amplitude sweep testing. The rheometer measurement plate waited 1 minute upon finding contact to allow for structural recovery. 25 data points were collected via a logarithmic ramp from 0.01% to 100% shear strain at a constant angular frequency of 10 1/s.

For viscosity tests, slurries were prepared in different ways to probe the effects of sonication and heating on the slurries. A base slurry formulation was first prepared by mixing ball-milled algae powder into deionized water (non-treated, NT). From this baseline, three additional conditions were generated: (i) sonicated (S; NT followed by sonication), (ii) heated (H; NT followed by heating at 80 °C for 10 min in an autoclave), and (iii) sonicated and heated (S+H; sonication followed by heating under the same conditions). The rheometer plate was held at 25 ± 0.2 °C with a 1 minute surveillance time to ensure constant temperature throughout the test. 21 data points were collected via a logarithmic ramp from 0.1 to 100 1/s. Data at each point was recorded after the viscosity reached a steady state or after 30 seconds, depending on which came first.

4.1.9 X-Ray Fluorescence (XRF)

Elemental composition of the algae powders was measured using a Bruker M4 Tornado (Bruker, Billerica, MA, USA) XRF equipped with a Rh anode tube. Ten distinct measurements were taken of the ball-milled algae powder and the results were averaged to distill elemental compositions representative of the bulk biomass used herein.

4.1.10 Carbon-Hydrogen-Nitrogen (CHN) Analysis

Dried, ground, and pre-weighed *U. lactuca* powder was prepared for CHN analysis by acidification to remove inorganic carbonates²⁴². Each sample was dosed (dropwise) with 6% sulfuric acid and then placed in a drying oven for 30–60 min. This process was repeated until samples no longer reacted to the acid, i.e., until no inorganic carbonates remained. Next, dry samples were enclosed in silver capsules and evaluated using a CEC 440HA elemental analyzer (Exeter Analytical, North Chelmsford, MA, USA). Analysis of the carbon, hydrogen, and nitrogen content of the *U. lactuca* powder was performed using the Dumas combustion method.

4.1.11 Biodegradation

The biodegradation experiment was conducted in reaction vessels sealed from the outside environment. Within each vessel, 1 g of ground-up sample was added to 500 mL of natural seawater inoculum prepared in accordance with ASTM D6691-17. Natural seawater collected from Elliot Bay near Seattle, WA, USA was supplied by Seattle Aquarium to be used as the base of the inoculum. The pH of the seawater collected on May 27, 2025, was estimated to be 7.9–8.0 based on monitoring data from the local station between May 25 and June 01, 2025. Inorganic nutrients in the form of NH_4Cl and $\text{KH}_2(\text{PO}_4)$ were added to the natural seawater at concentrations of 0.5 g/L and 0.1 g/L respectively to form the seawater inoculum. 100 mL of 3 M NaOH solution was used as a CO_2 scrubber for each reaction vessel. The reaction vessels were controlled at 30 °C, and connected to oxygen bags, with real-time oxygen consumption monitored using a BPC Blue closed-loop apparatus (BPC Instruments AB, Lund, Sweden). Microcrystalline cellulose was used as the

reference material and several reactors were prepared with inoculum alone as blanks. Triplicate tests were performed for the *U. lactuca* foams, cellulose reference, and the blanks. The consumed oxygen was recorded as the Biochemical Oxygen Demand (BOD_s) in grams of O₂.

Biodegradation is calculated as:

$$D_t = \frac{BOD_s}{ThOD} * 100 \quad (4.1)$$

The theoretical oxygen demand (ThOD) is calculated using two different methods based on ISO 14851²⁴³ and described below. The first method used the measured elemental compositions from CHN and XRF. The oxygen content of *U. lactuca* was assumed to be the balance of CHN measurements and XRF measurements such that the total elemental composition sums to 100 wt.%. The ThOD equation from ISO 14851 is modified to include additional elements present in the algae and to account for the formation of nitrates during degradation rather than ammonia (Annex B, ISO 14851²⁴³). It is assumed that the additional monovalent and divalent ions in the algae form oxides and hydroxides:

$$ThOD = \frac{16}{M_r} [2c + 0.5(h - cl - br - 2mg - 2ca - sr) + 2.5n + zn + 1.5al + 1.5fe + 3s + 2.5p + 0.5(na + k) + 2 * mg + 2ca + 2sr - o] \quad (4.2)$$

This method assumes that the algae is composed of representative classes of “molecules” composed of 100 atoms, with the identity of these atoms determined by the elemental composition, expressed in mass% of dry algae. The relative molecular weight (M_r) is calculated using Eq. 4.3, where X is the percent of total mass of each constituent element and m_{ax} is its corresponding mass:

$$M_r = \Sigma(X * m_{ax}) \quad (4.3)$$

A summary of the assumptions used in this method are as follows. The concentration of oxygen in *U. lactuca* is calculated by subtracting the sum of the concentration by mass of

all other elements detected from CHN and XRF. The ThOD equation, Eq. 4.2, is adjusted to account for nitrification rather than the formation of ammonia as is prescribed by Annex B of ISO 14851. ISO 14851 predicts the formation of sodium oxide but does not account for the formation of potassium oxide and accounts for the formation of HCl but not of other hydrogen halides. The standard also does not account for the formation of bivalent inorganic oxides. Literature suggests that these may react with hydrogen and precipitate in seawater. Thus, bromine, magnesium, calcium, strontium, zinc, aluminum, iron, and potassium were included in Eq. 4.2. Further analysis of the degradation products and residue would be required to verify these assumptions.

The second method is based on the macromolecular composition of *U. lactuca*. In this method, the composition of lipids, uronic acid, amino acids, and monosaccharides were taken from Yaich et. al.²⁴⁴, carotenoids and chlorophyll compositions from El-Din²⁴⁵, and inorganics from XRF data presented in this paper. The ThOD for each macromolecule was calculated using Eq. 4.4:

$$ThOD = \frac{16[2c + 0.5h + 2.5n + 3s - o]}{M_r} \quad (4.4)$$

4.1.12 Thermogravimetric Analysis (TGA)

TGA was used to determine the ash content of the ball-milled *Ulva* powders. Tests were started in an air atmosphere. Temperature was first ramped from room temp to 105 °C at a rate of 10 °C/min, before an isothermal hold of 20 minutes. Then, the temperature was ramped at 10 °C/min to 250 °C, where it was held for another 30 minutes. Next, the temperature was ramped at 10 °C/min to 575 °C, where it was held for 720 minutes. Finally, the environment was switched to a nitrogen environment and the temperature was ramped at 20 °C/min to 900 °C, where it was held for 10 minutes. The percent of weight at the end of the 575 °C isotherm in air was determined to be the ash content of the biomass.

4.1.13 Pycnometry

Pycnometry tests were carried out using an AccuPyc II 1340 (Micromeritics, Norcross, Georgia, USA) in a helium atmosphere with approximately 2 grams of ball-milled algae powder per test. 10 data collection cycles were run per test and reported values are an average of the density results from those 10 runs. Samples were not dried before each experiment because the drying process had a negligible effect on the average density results.

4.1.14 Porosity

Apparent density of each sample was measured by using calipers to measure the height and diameter of each sample, calculating the volume, and dividing it by the sample mass. Porosity was then calculated as:

$$1 - \frac{\text{apparent density}}{\text{powder density}} \quad (4.5)$$

where powder density is the density of the powder that makes up the sample as measured by pycnometry.

4.1.15 Flammability

The flammability of samples were tested using a modified UL94-HB setup. Acoustic foam samples were cut into approximately 5 mm thick slices. Samples were placed with their widest face on a pair of large tweezers to keep the samples elevated with no platform directly underneath them. They were brought into contact with an open flame for 60 seconds before being pulled away from the flame. The time to self-extinction was measured for each sample.

4.1.16 Sound absorption measurements

The sound absorption performance of the algae-based porous samples was evaluated using a standardized impedance tube setup (AcoustiTube, Gesellschaft für Akustikforschung Dresden mbH, Dresden, Germany) as seen in Figure 4.1. The system is designed to measure the normal-incidence sound absorption coefficient, reflection factor, and impedance ratio

of materials in a controlled laboratory environment. Measurements were conducted based on the transfer-function method described in DIN EN ISO 10534-2 and ASTM E1050.

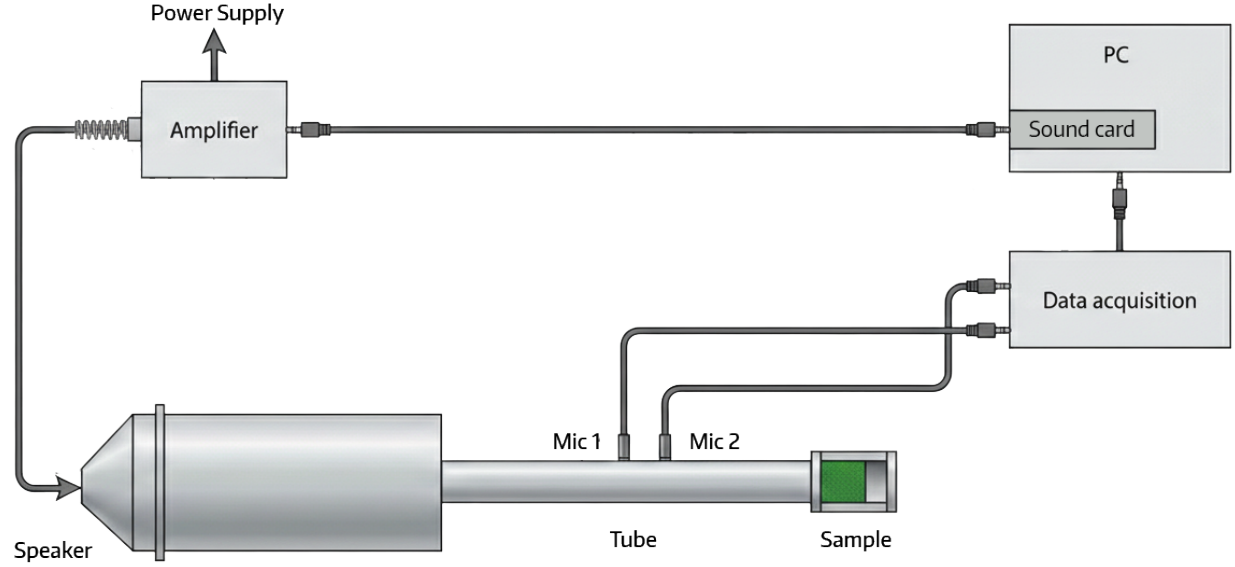


Figure 4.1: Schematic of the impedance tube setup for normal-incidence sound absorption measurements.

The impedance tube had an internal diameter of 40 mm, defining the valid frequency range for plane-wave propagation. A two-microphone transfer-function method was employed to determine the acoustic response. Each sample was mounted at the rigid termination of the tube, while broadband white noise was generated by a loudspeaker positioned at the opposite end. Two GRAS 40PH-10 microphones were used to capture the acoustic pressure field. These microphones offer a wide frequency response up to 20 kHz and a large dynamic range (from below 32 dB to 135 dB), ensuring accurate acquisition of both low- and high-amplitude signals. The microphones were positioned at predefined locations along the tube to enable simultaneous measurement of incident and reflected sound waves. The recorded signals were processed using the transfer-function approach to compute the normal-incidence sound absorption coefficient over the frequency range of interest. This method enables the decomposition of the sound field into incident and reflected components, providing a reliable characterization of the material's acoustic behavior. The resulting experimental data serve as a basis for validating and calibrating the analytical and surrogate models developed in this study.

4.1.17 Material properties from acoustic measurements

This stage involves calibrating the analytical model and performing a parametric analysis to evaluate the acoustic behavior of the algae-based porous samples. The experimental measurements obtained from the impedance tube and laboratory characterization are combined to estimate the effective material properties through an inverse modeling approach. A Genetic Algorithm (GA) optimization framework was employed to fit the experimentally measured absorption coefficients to the Johnson–Champoux–Allard (JCA) model²⁴⁶. By linking measurable acoustic responses to microstructural parameters, the model enables reliable identification of material properties for bio-based materials with complex and irregular morphologies²⁴⁷.

The JCA model characterizes the acoustic behavior of porous media using five key parameters:

- **Porosity** (ϕ) – ratio of void volume to total volume.
- **Flow resistivity** (σ) – resistance to steady airflow through the material.
- **Tortuosity** (α_∞) – complexity of pore pathways.
- **Viscous characteristic length** (Λ) – associated with viscous losses.
- **Thermal characteristic length** (Λ') – governs thermal exchanges.

The effective density $\rho(\omega)$ is defined as:

$$\rho(\omega) = \rho_0 \alpha_\infty \left[1 + \frac{\sigma \phi}{i \omega \rho_0 \alpha_\infty} \sqrt{1 + i \frac{4 \alpha_\infty^2 \eta \rho_0 \omega}{\sigma^2 \Lambda^2 \phi^2}} \right] \quad (4.6)$$

where $\omega = 2\pi f$ is the angular frequency, $\rho_0 = 1.21 \text{ kg/m}^3$ is the density of air, i is the imaginary unit, and $\eta = 1.84 \times 10^{-5} \text{ Pa} \cdot \text{s}$ is the dynamic viscosity of air.

The effective bulk modulus $K(\omega)$ is defined as:

$$K(\omega) = \gamma P_0 \left[\gamma - (\gamma - 1) \left(1 + \frac{8 \eta}{i \omega \rho_0 \Lambda'^2 \text{Pr}} \sqrt{1 + i \frac{\omega \rho_0 \text{Pr} \Lambda'^2}{16 \eta}} \right)^{-1} \right]^{-1} \quad (4.7)$$

where $\gamma = 1.4$ is the ratio of specific heats, $P_0 = 101325 \text{ Pa}$ is the atmospheric pressure, and $\text{Pr} = 0.77$ is the Prandtl number.

From equations 4.6 and 4.7, the wave number $k(\omega)$, characteristic impedance $Z_c(\omega)$, surface impedance $Z_s(\omega)$, and normal-incidence absorption coefficient $\alpha(\omega)$ are computed.

$$k(\omega) = \omega \sqrt{\frac{\rho(\omega)}{K(\omega)}} \quad (4.8)$$

$$Z_c(\omega) = \sqrt{\rho(\omega) K(\omega)} \quad (4.9)$$

$$Z_s(\omega) = -i Z_c(\omega) \cot(k(\omega) d) \quad (4.10)$$

$$\alpha(\omega) = 1 - \left| \frac{Z_s(\omega) - Z_0}{Z_s(\omega) + Z_0} \right|^2 \quad (4.11)$$

where d is the sample thickness, and $Z_0 = 415 \text{ Pa}\cdot\text{s}/\text{m}$ is the characteristic impedance of air. To determine the material parameters, the GA minimizes the squared error between the measured and predicted absorption coefficients. Since the measured sample thicknesses varied slightly across specimens (Table 4.1), the average thickness was used in the optimization process.

The optimization was performed using a population-based search with 1,000 individuals over 50 generations, including 200 elite individuals retained at each generation. A mutation probability of 0.006 was applied to maintain diversity within the population. As discussed in Section 4.1.14 and 4.1.7, porosity (ϕ) and tortuosity (α_∞) were obtained from the laboratory measurements and treated as nearly fixed inputs, only being allowed to deviate from their averaged measurements by the standard deviation. The flow resistivity, viscous characteristic length, and thermal characteristic length were identified through the optimization process and their bounds were defined based on physically realistic ranges. Following calibration, an acoustic comparison and parameter analysis were conducted using the fitted JCA models. In this stage, all samples were evaluated at a uniform thickness

Sample name	Species	Concentration (%)	Sample number	Thickness (mm)
ulva_lactuca_5_1	<i>U. lactuca</i>	5	1	21.92
ulva_lactuca_5_2	<i>U. lactuca</i>	5	2	21.81
ulva_lactuca_5_3	<i>U. lactuca</i>	5	3	22.97
ulva_lactuca_7.5_1	<i>U. lactuca</i>	7.5	1	25.45
ulva_lactuca_7.5_2	<i>U. lactuca</i>	7.5	2	24.34
ulva_lactuca_7.5_3	<i>U. lactuca</i>	7.5	3	25.24
ulva_lactuca_10_1	<i>U. lactuca</i>	10	1	25.31
ulva_lactuca_10_2	<i>U. lactuca</i>	10	2	26.40
ulva_lactuca_10_3	<i>U. lactuca</i>	10	3	25.41
ulva_ohnoi_5_1	<i>U. ohnoi</i>	5	1	22.05
ulva_ohnoi_5_2	<i>U. ohnoi</i>	5	2	20.90
ulva_ohnoi_5_3	<i>U. ohnoi</i>	5	3	22.90
ulva_ohnoi_7.5_1	<i>U. ohnoi</i>	7.5	1	22.90
ulva_ohnoi_7.5_2	<i>U. ohnoi</i>	7.5	2	24.88
ulva_ohnoi_7.5_3	<i>U. ohnoi</i>	7.5	3	23.27
ulva_ohnoi_10_1	<i>U. ohnoi</i>	10	1	24.81
ulva_ohnoi_10_2	<i>U. ohnoi</i>	10	2	25.05
ulva_ohnoi_10_3	<i>U. ohnoi</i>	10	3	24.68
ulva_sp_5_2	<i>U. sp</i>	5	2	23.93
ulva_sp_5_3	<i>U. sp</i>	5	3	23.62
ulva_sp_7.5_2	<i>U. sp</i>	7.5	2	23.93
ulva_sp_7.5_3	<i>U. sp</i>	7.5	3	23.81
ulva_sp_10_2	<i>U. sp</i>	10	2	25.96
ulva_sp_10_3	<i>U. sp</i>	10	3	25.04

Table 4.1: All the measured samples, organized by name, species, concentration, sample number and thickness

of 25 mm to enable direct comparison of their acoustic performance. By isolating thickness effects, the influence of intrinsic material parameters on sound absorption could be systematically assessed. To provide a practical evaluation of the acoustic performance, the mean Noise Reduction Coefficient (NRC) was calculated for all samples based on the ASTM C423 standard²⁴⁸, defined as the average absorption coefficient at 250, 500, 1000, and 2000 Hz. All samples, at a thickness of 25 mm and alongside a commercial acoustic absorber were compared using the mean NRC.

4.2. Results

4.2.1 Algae composition and characterization

To understand the properties of our final materials, we first need to examine the composition and structure of our feedstock. The composition of algae can change significantly based on the species, growth environment, and season, so here we use a combination of TGA, XRF, and FTIR to present brief compositional descriptors of our feedstock materials.

TGA curves of the ball-milled powders are shown in Figure 4.2a. The mass loss after the isothermal hold at 105 °C shows that the ball-milled powders have similar moisture contents of 12.8%, 14.9%, and 12.2% for *U. lactuca*, *U. ohnoi*, and *U. sp*, respectively.

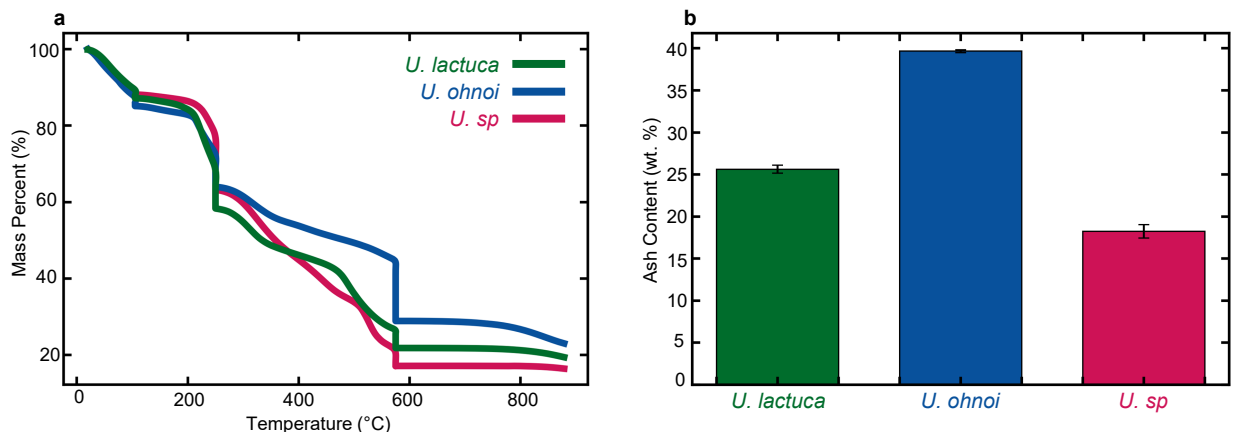


Figure 4.2: **TGA of *Ulva* species.** (a) TGA and (b) ash content of *U. lactuca*, *U. ohnoi*, and *U. sp*.

Degradation onset temperatures, defined here as the temperature at which the mass drops 1% from the value at the end of the 105 °C isotherm, are slightly more varied. *U. lactuca* has a degradation onset temperature of 160 °C, *U. ohnoi* begins degrading at 152 °C, and *U. sp* starts to degrade at 171 °C. After the 250 °C hold, each sample varies significantly in its degradation profile, indicating differences in composition of more thermally stable carbohydrates, proteins, and/or aromatics between the three species. Finally, the ash content, measured after the 575 °C hold, was 25.62%, 39.66%, and 18.24% for *U. lactuca*, *U. ohnoi*, and *U. sp*, respectively (Figure 4.2b). These differences in ash indicate variations in inorganic content such as silicates and minerals.

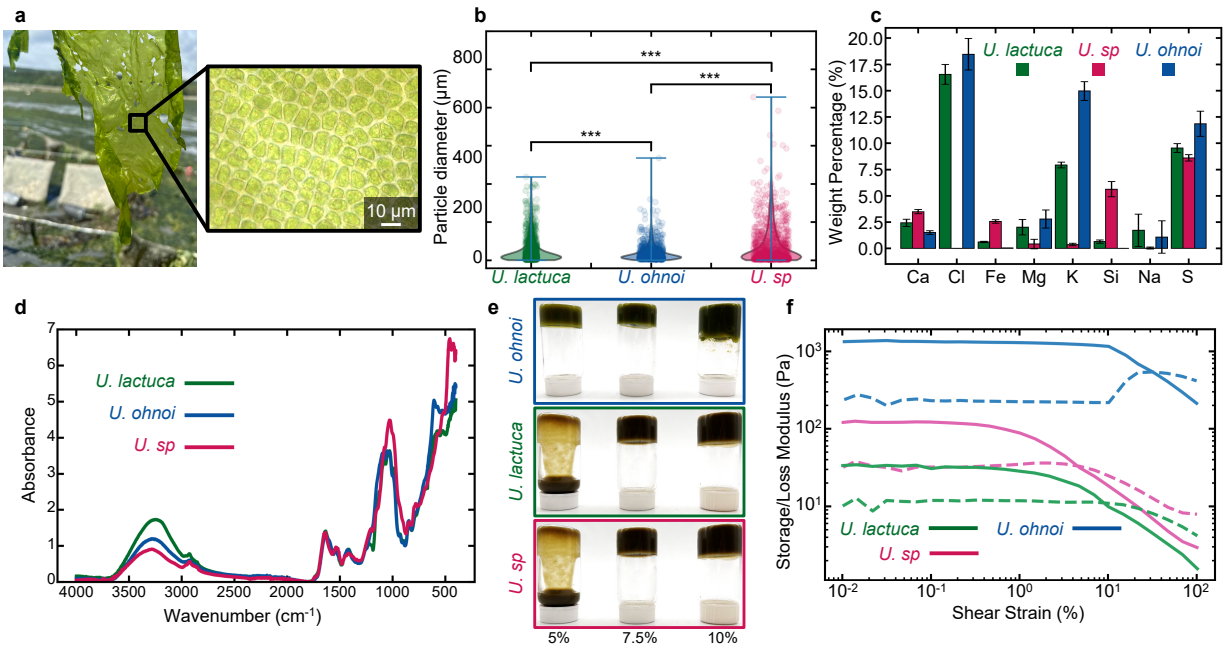


Figure 4.3: **Morphology, composition, and processing of *Ulva*.** **a**, Photograph and optical microscopy of *Ulva sp.* **b**, Particle size distributions of ball-milled *Ulva* powder. **c**, Comparison of the composition of three *Ulva* species measured by XRF. **d**, FTIR comparison of the three *Ulva* species. **e**, Viscosity versus shear rate curves of *Ulva sp.* at different concentrations in water. **f** Storage and loss moduli of three **Ulva** species at 7.5 wt.%.

To further investigate these differences, an elemental analysis of each species of algae was done via XRF (Figure 4.3c). *U. sp* shows no detectable chlorine, in contrast to *U. lactuca* (18.5%) and *U. ohnoi* (16.5%). This is consistent with the elevated magnesium (2.0, 2.8%), potassium (7.9, 15.0%), and sodium (1.1, 1.7%) in *U. lactuca* and *U. ohnoi* respectively, indicative of retained chloride salts, whereas *U. sp* contains <0.5% of each. In contrast, *U. sp* is enriched in calcium (3.5%) and iron (2.6%) relative to *U. lactuca* (2.4% calcium, 0.6% iron) and *U. ohnoi* (1.5% calcium, 0.04% iron), suggesting association with non-chloride phases. It also exhibits elevated silicon (5.6% vs. 0.7% for *U. lactuca* and 0% for *U. ohnoi*), consistent with diatom and/or sand contributions, in agreement with FTIR (Figure 4.3d) and morphological observations (Figure 4.12). The absence of chloride salts in *U. sp* likely reflects more extensive washing during preparation. Finally, XRF shows slight differences in sulfur content between the species, with *U. sp* having the lowest at 8.6%, *U. ohnoi* having the most at 11.9%, and *U. lactuca* being in the middle with 9.5%. Sulfur is largely present in *Ulva* in ulvans, the dominant sulfated polysaccharide of *Ulva*, which is unique to the *Ulva* genus, as well as in amino acids such as cysteine. Given

that the protein content for *Ulva* is generally quite low (between 3 and 26% depending on species, growth environment, and harvest time^{249,250}), these differences likely correspond to differences in the amount of ulvan between the species.

We can further explore the differences in polysaccharide and protein composition between the species through FTIR. Complete assignment of the IR peaks can be seen in Table 4.2. The broad band at 3250-3290 cm^{-1} arises from overlapping O–H and N–H stretching modes, associated with polysaccharides, bound water, and proteins. Its high intensity and breadth indicates a large variety of hydrogen bonding states present in these samples, suggesting that O–H-rich polysaccharides and hydrogen-bonded water dominate this region, although N–H contributions cannot be excluded. *U. lactuca* shows the highest intensity and a slight shift of the main peak to lower wavenumber (3248 cm^{-1}) relative to *U. ohnoi* and *U. sp* (3288 and 3286.5 cm^{-1}), indicating a higher carbohydrate content and potentially greater contribution from protein-associated N–H vibrations. The C–H stretching region associated with lipids and carbohydrates ($\sim 2925\text{--}2930$ cm^{-1}) follows a similar trend, with *U. lactuca* exhibiting higher intensity than the other two species.

Protein-associated amide bands show more subtle variation (Figure 4.4a). Amide I intensity (1550 cm^{-1} –1800 cm^{-1}) is comparable for *U. lactuca* and *U. sp*, and lower for *U. ohnoi*, while Amide II (1500 cm^{-1} –1550 cm^{-1}) is more pronounced in *U. sp*. These differences suggest modest variation in protein content and/or secondary structure but do not indicate clear systematic trends.

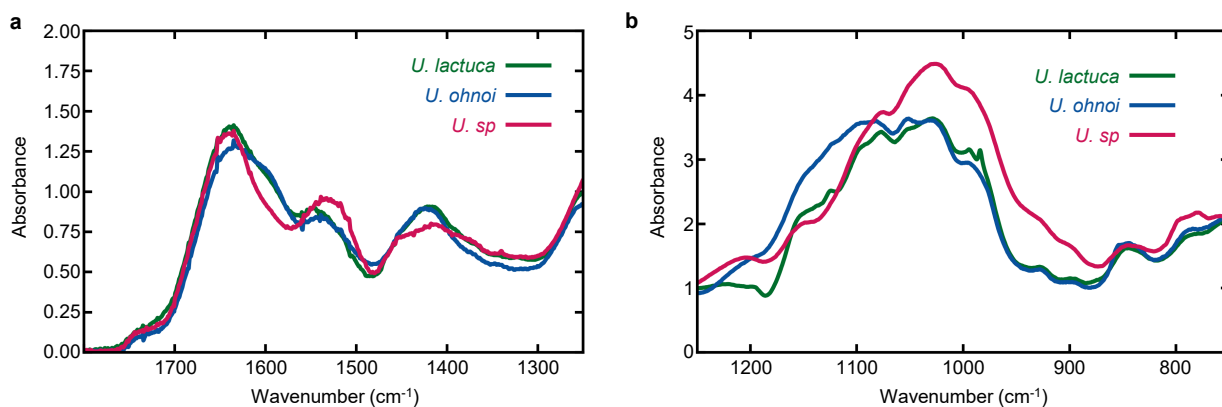


Figure 4.4: **Comparison of peaks in FTIR.** FTIR spectroscopy of *U. ohnoi*, *U. lactuca*, and *U. sp* in (a) the amide region (1800-1300 cm^{-1}) and (b) the polysaccharide region (1200-800 cm^{-1}).

Peak (cm ⁻¹)	Attribution	Source
3029-3639	Water $\nu(\text{O-H})$ stretching, protein $\nu(\text{N-H})$ stretching	Ref ²⁵¹⁻²⁵⁴
2800-3000	$\nu(\text{C-H})$ stretching of saturated hydrocarbons and alkane ethers	Ref ^{170, 251-255}
1712-1763	$\nu(\text{C=O})$ stretching in cellulose and fatty acid esters	Ref ^{251, 252, 255, 256}
1583-1709	Amide I band for proteins, $\nu(\text{C=O})$ stretching	Ref ^{170, 251-256}
1480-1580	Amide II band, $\delta(\text{N-H})$ of amides from proteins	Ref ^{251, 252, 255, 256}
1300-1480	CH_2 and CH_3 from methyl and methylene groups, bending of CH in acyl chains of lipids	Ref ^{251, 253-256}
1125-1175	C-O, C-C, and C-O-C in polysaccharides	Ref ^{251, 255, 257}
1100-1150	$\nu(\text{C-O-C})$ glycosidic bond	Ref ^{251, 255, 257}
1050-1100	$\nu(\text{Si-O})$ of silicates, $\nu(\text{C-O})$ of polysaccharides	Ref ^{251, 255, 256}
1000-1030	C-O and C-O-H alcohols and esters in polysaccharides	Ref ^{171, 253}
950-1000	CH_3 rocking, C=C symmetrical bending	Ref ²⁵⁸
820-880	Sulfates (C4-O-S), CH bending in glucose/galactose, C-O-SO ₃	Ref ^{171, 259}
575-650	Sulfides (C=S)	Ref ²⁵⁴

Table 4.2: *Ulva* FTIR Band Attributions

Polysaccharides are generally characterized at peaks between wavenumbers 800 cm^{-1} and 1200 cm^{-1} , but for algae, it can be difficult to draw conclusions due to the presence of silicates from diatoms or sand. *U. sp* has significantly more silicon than the other two species, as shown from the XRF data. In the FTIR, we see a significant increase in intensity for *U. sp* at 1027.5 cm^{-1} , and in general the *U. sp* triplet of peaks is higher than that of the other two species. While literature typically gives a peak for silicates at around 1075 cm^{-1} , *U. sp*'s peak at that position is only slightly higher than that of the other two species. *U. lactuca* does not have a distinct peak at that location, but rather has a broad peak centered at around 1100 cm^{-1} . *U. ohnoi* has a more pronounced peak at that location, but it is lower in intensity. Given the lack of silicon present in *U. lactuca* and *U. ohnoi* and the relative abundance of silicon in *U. sp* (Figure 4.3c), it may be that the silicates are showing up at 1027.5 cm^{-1} more so than at 1075 cm^{-1} . Indeed, silicates can have broad peaks in the range of 900 to 1100 cm^{-1} depending on bonding environment, so for *U. sp* specifically, we can expect a mix of contributions from silicates and polysaccharides between this range²⁶⁰⁻²⁶². *U. ohnoi* has a higher absorbance between 1082 and 1180 cm^{-1} , which is typically a region attributed to C-O, C-C, and C-O-C bonds in polysaccharides^{251,255}. Murdock and Wetzel attribute these peaks to starch, although these bonds appear in ulvans, cellulose, and other polysaccharidic macromolecules^{171,263}. Still, optical microscopy comparisons between the three species (Figure 4.5) show starch granules within the cells of *U. ohnoi* only²⁶⁴⁻²⁶⁷. The abundance of these starch granules could explain the higher intensity in the FTIR spectrum between 1082 and 1180 cm^{-1} . It is known that, when grown in low-nutrient conditions, green algae will accumulate starch at the expense of lipids and/or proteins, which may also explain the lower intensity at the Amide I and II bands for *U. ohnoi*.

The peak at 1125 cm^{-1} attributed to $\nu(\text{C-O-C})$ of glycosidic bonds in polysaccharides, marks another clear difference between the three species. While *U. lactuca* has a pronounced peak, *U. ohnoi* has a higher intensity but less well-defined peak and *U. sp* shows no peak in that wavenumber. Thus, *U. ohnoi* likely has more polysaccharidic content than the other two species, and *U. sp* may have the least amount of polysaccharides

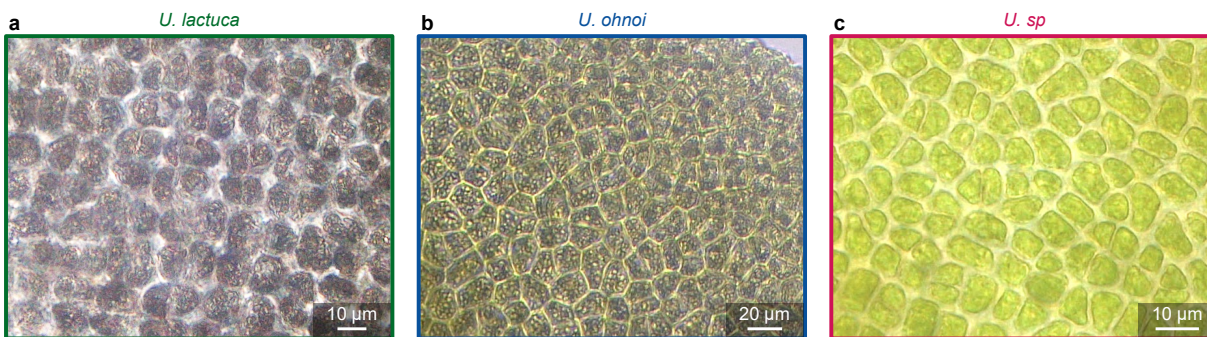


Figure 4.5: **Optical microscopy of *Ulva* species.** Optical microscopy showing cells of (a) *U. lactuca*, (b) *U. ohnoi*, and (c) *U. sp.*

out of the three species. The peak centered around 845 cm^{-1} represents sulfates and C-H bending in glucose/galactose, indicative of ulvan monomers. We see that all species have a similar intensity at this peak, but *U. ohnoi* and *U. lactuca* have more pronounced peaks, while *U. sp* has a broader peak. Similarly, at the peak between 550 cm^{-1} and 650 cm^{-1} , *U. ohnoi* has a very prominent peak with higher intensity, *U. lactuca* has a more rounded peak at a lower intensity, and *U. sp* does not show a peak at all. This peak corresponds with sulfides (C=S), which, along with the previously mentioned sulfates, are present in the sulphated polysaccharides characteristic of *Ulva* species.

Taken together, we see that *U. ohnoi* has distinct peaks and higher intensities at wavenumbers corresponding to sulfides and polysaccharides. *U. sp* generally has lower polysaccharidic content, but it has a higher presence of silicates that can make it difficult to get an accurate analysis of the main polysaccharide peaks. *U. lactuca* is generally more similar to *U. ohnoi*, but falls between *U. ohnoi* and *U. sp* in the main protein and polysaccharide peaks. However, *U. lactuca* has much higher lipids and -OH/N-H peaks, potentially indicating more lipid content as well as more hydrogen bonding, which could reflect the presence of polysaccharides but could also be obscured by the presence of bound water in the samples.

4.2.2 Algae slurry and foam characteristics

Algal foams were prepared by dispersing ball-milled powders in water followed by freeze-drying. Ball-milling created particle sizes, as measured by SEM, on the order of tens

of micrometers, with median particle sizes of 23 μm , 22 μm , and 30 μm for *U. lactuca*, *U. ohnoi*, and *U. sp*, respectively (Figure 4.3b). *U. sp* exhibited a broader particle size distribution, including particles greater than 600 μm , while *U. lactuca* and *U. ohnoi* were largely confined below 400 μm .

Manual mixing yielded heterogeneous slurries; probe sonication improved dispersion and resulted in reproducible rheological behavior (Figure 4.6, non-processed versus sonicated). Sonication also induced gelation in all compositions except *U. lactuca* and *U. sp* at 5 wt.% (Figure 4.3e), which was not observed in non-processed slurries. Although sonication introduced measurable heating, control experiments isolating thermal effects showed that heating alone reduced viscosity and did not reproduce the gelation or rheological behavior of sonicated samples, indicating that mechanical homogenization rather than temperature is the primary driver for the observed rheological behavior of the slurries (Figure 4.6).

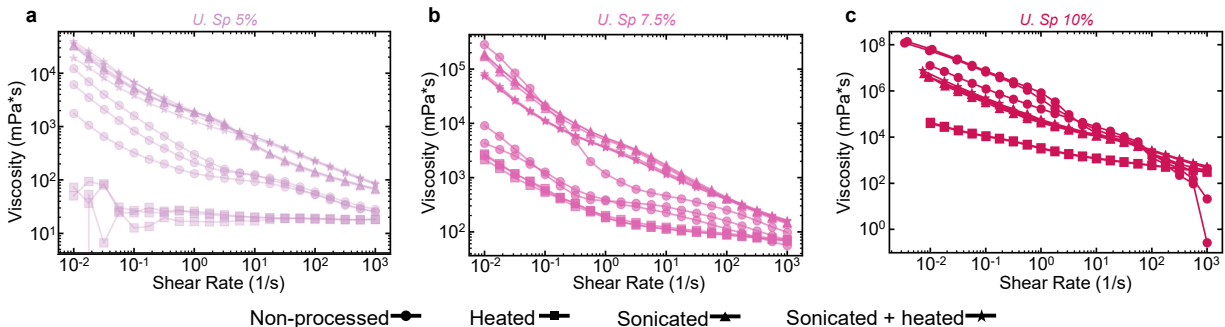


Figure 4.6: **Flow curves for *U. sp*.** Viscosity differences of *U. sp* at (a) 5 wt.%, (b) 7.5 wt.%, and (c) 10 wt.% in DI water with either sonication, heating, sonication and then heating, or non-treated.

Oscillatory rheology (Figure 4.3f and Figure 4.7) revealed strong species-dependent behavior. All species at all concentrations have a storage modulus higher than their loss modulus, demonstrating that the sonicated slurries are viscoelastic gels with solid-like behavior. *U. ohnoi* exhibited the highest storage moduli at 5 and 7.5 wt.% (567 ± 34 Pa and 1378 ± 98 Pa), compared to substantially lower values for *U. sp* (25 ± 1 and 121 ± 3 Pa) and *U. lactuca* (16 ± 10 and 44 ± 14 Pa). *U. sp* and *U. lactuca* at 5 wt.% are the only gels that did not visually have a solid-like character after sonicating (see Figure 4.3e) and were also the only gels with storage moduli below 30 Pa. At 10 wt.%, both *U. ohnoi* and

U. sp showed high stiffness (4875 ± 863 and 5698 ± 289 Pa), while *U. lactuca* remained significantly lower (239 ± 19 Pa). *U. ohnoi* consistently exhibited the highest crossover strain, indicating greater resistance to yielding, although sharp transitions from the linear viscoelastic plateau to the flow region indicate either abrupt network failure or apparent plate slippage at high shear rates, complicating interpretation at those conditions. This abrupt transition in storage modulus was also apparent for *U. sp* at 10 wt.%, but not for other concentrations or for any *U. lactuca* samples.

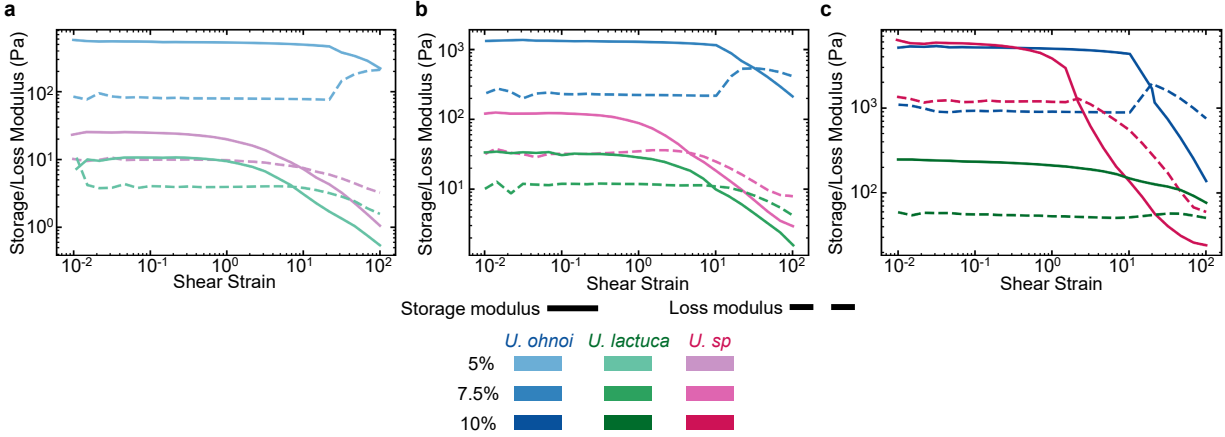


Figure 4.7: **Rheological amplitude sweeps for *Ulva*.** Storage and loss modulus for *U. ohnoi*, *U. lactuca*, and *U. sp* at (a) 5 wt.%, (b) 7.5 wt.%, and (c) 10 wt.% concentrations in DI water.

The transition in the loss modulus, however, is different for *U. sp* at 10 wt.% than from *U. ohnoi* at all concentrations, lacking a distinct increase in loss modulus as it approaches the crossover strain. This indicates that *U. sp* 10 wt.% gels undergo network failure at low shear strains with little dissipative effects from the gel's microstructure while *U. ohnoi* gels undergo a less brittle failure with some dissipative effects due to rearrangement of the gel network. This behavior aligns with compositional differences: ulvan-rich species (*U. ohnoi*, *U. lactuca*) can form ionically crosslinked networks, whereas *U. sp*, with lower sulfur content and reduced salt levels, lacks the necessary components for gelation. Additionally, the starch present in *U. ohnoi* likely contributes to the elastic character of the gel and may contribute to slipping as well.

Morphological analysis

Foam morphology was characterized by SEM across all species and concentrations (Figure 4.12, Figures 4.8-4.10). All samples consist of collapsed algal sheets forming porous networks, with species-dependent differences in sheet integrity and connectivity. *U. sp* forms matrices made out of large, relatively intact sheets with preserved cellular imprints (see Figure 4.10e). These structures are densely packed at 10 wt.%, with limited inter-sheet porosity.

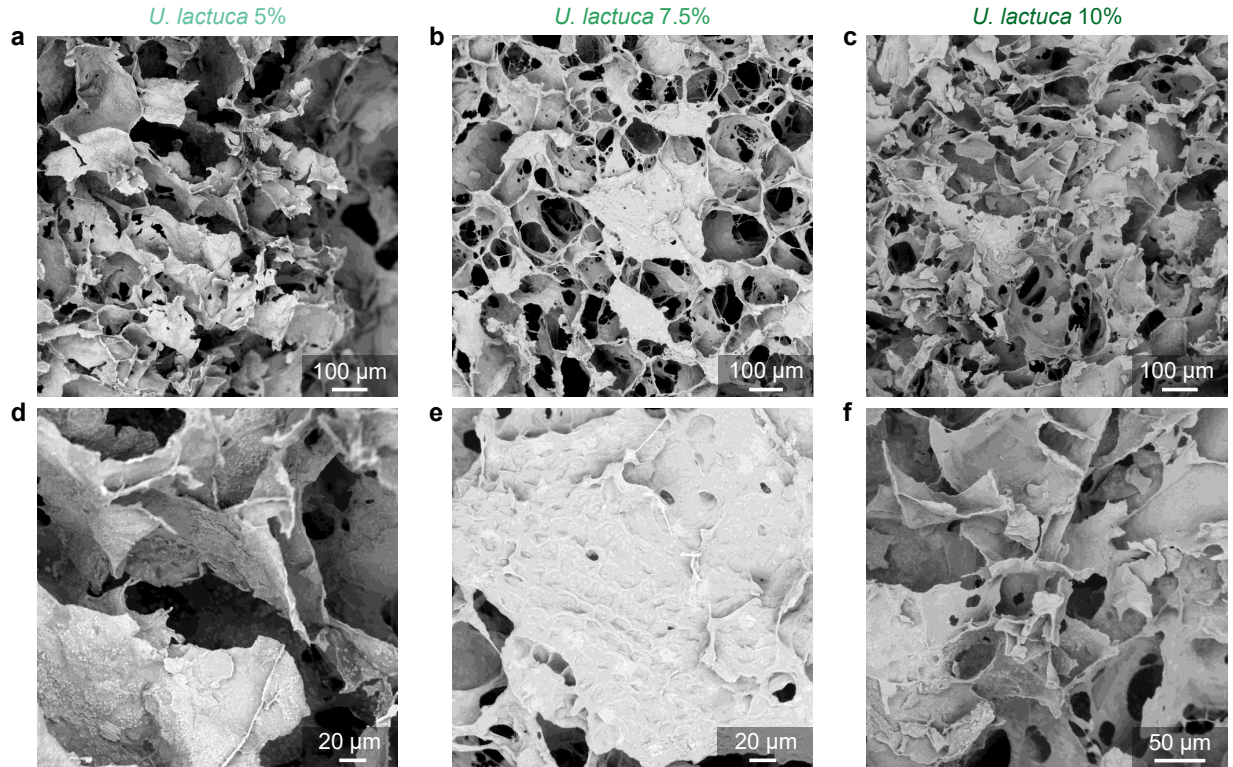


Figure 4.8: **SEM of *U. lactuca*.** SEM of *U. lactuca* at (a) 5 wt.%, (b) 7.5 wt.%, and (c) 10 wt.% concentrations, and (d-f) their respective high-magnification images.

In contrast, the 7.5 wt.% samples show reduced packing density and smaller flake sizes relative to both 5 and 10 wt.%, indicating non-monotonic structural evolution with concentration. Residual diatoms are observed in *U. sp* (Figure 4.11), consistent with compositional analysis, and are absent in the other species, likely due to the use of more thorough industrial harvesting and cleaning methods.

U. lactuca and *U. ohnoi* exhibit related morphologies characterized by sheet-based networks with concentration dependent connectivity. At 5 wt.%, *U. ohnoi* retains larger,

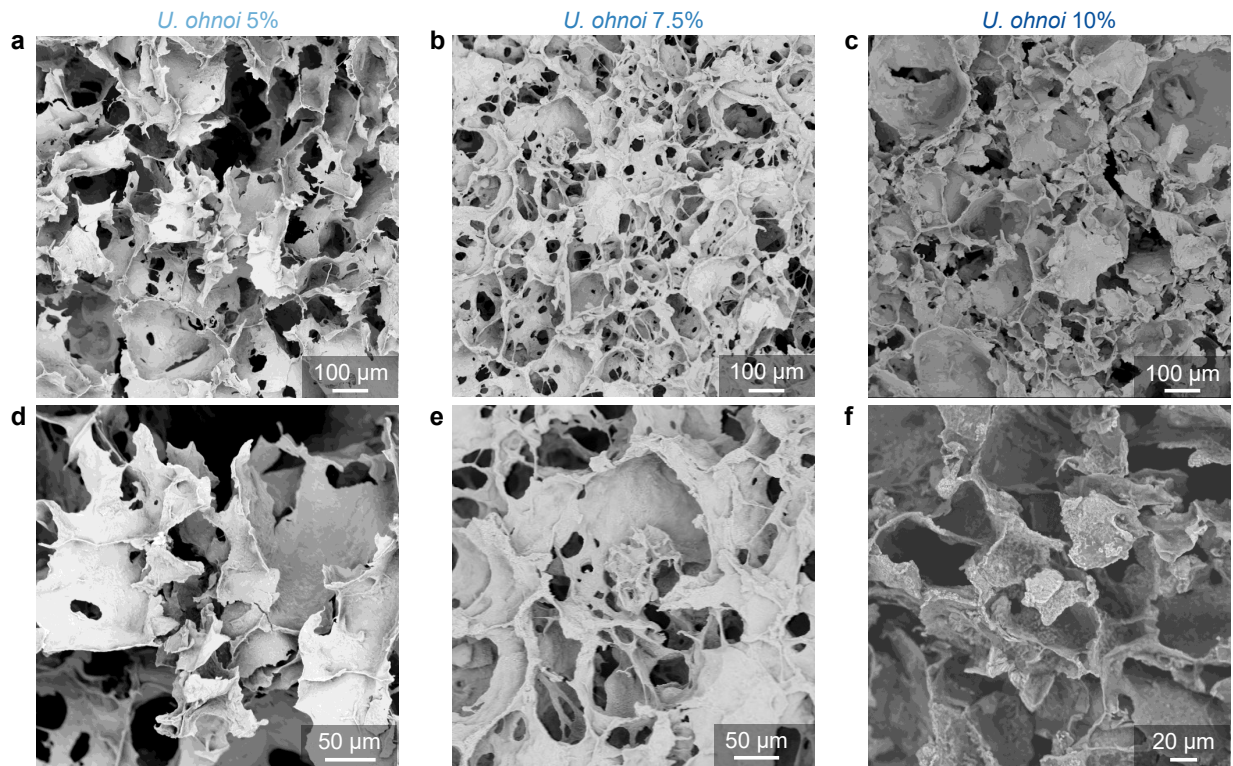


Figure 4.9: **SEM of *U. ohnoi***. SEM of *U. ohnoi* at (a) 5 wt.%, (b) 7.5 wt.%, and (c) 10 wt.% concentrations, and (d-f) their respective high-mag images.

more intact sheets, whereas *U. lactuca* appears more fragmented, with smaller, collapsed domains. At 7.5 wt.%, both species develop interconnected, web-like structures with fibrillar bridges between adjacent domains. While the overall architecture is similar, *U. lactuca* forms a more open network with larger pores, whereas *U. ohnoi* is comparatively denser, consistent with pore size analysis (Figure 4.12d). At 10 wt.%, the morphologies converge, with both systems dominated by densely packed, collapsed sheets and limited distinguishable differences. Across these structures, porosity arises from both freeze-drying-induced voids and perforations within the sheet-like matrix, indicating that network disruption contributes alongside ice templating. Surface salt residues are observed in *U. lactuca*, consistent with compositional analysis.

The distributions of pore diameters for all samples are right-skewed with the majority of pores below 50 μm . Median pore sizes for *U. lactuca* are 53, 64, and 44 μm , for *U. ohnoi* are 59, 36, and 53 μm and for *U. sp* are 44, 53, and 50 μm , for 5, 7.5, and 10 wt.% concentrations, respectively. Larger pores (>300 μm) are rare and observed only in *U.*

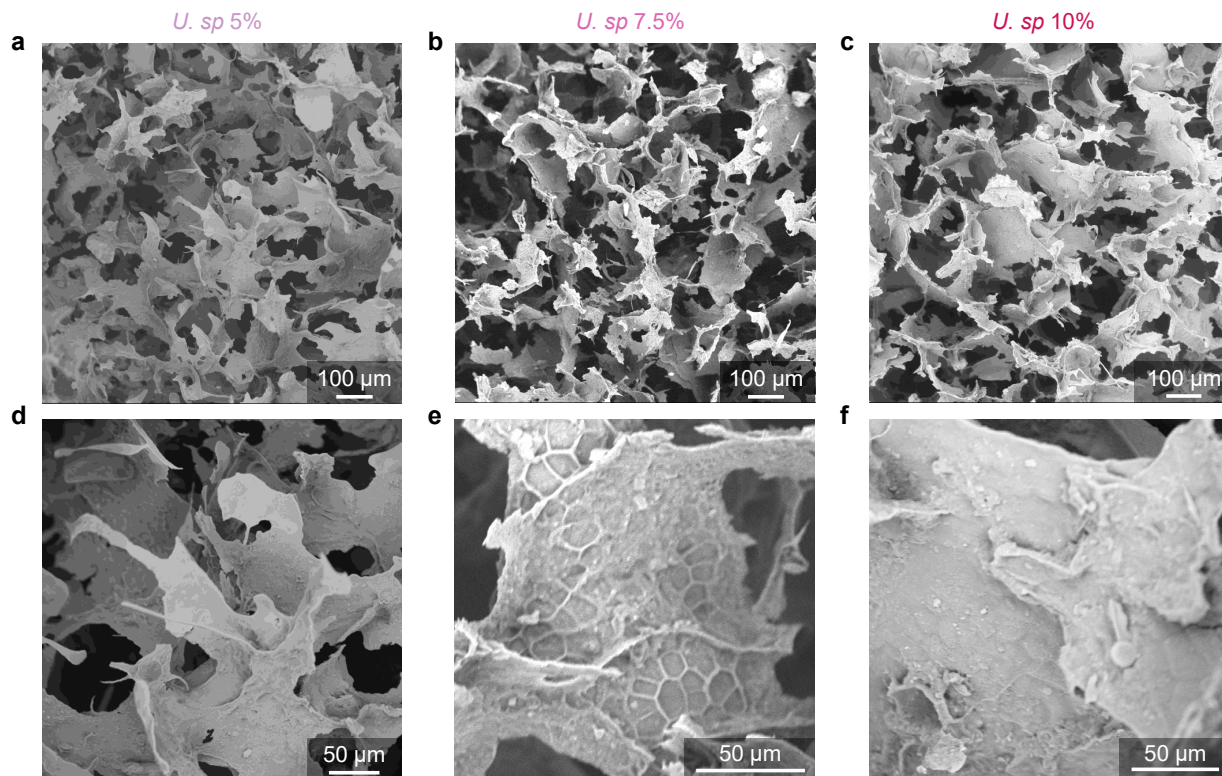


Figure 4.10: **SEM of *U. sp.*** SEM of *U. sp.* at (a) 5 wt.%, (b) 7.5 wt.%, and (c) 10 wt.% concentrations, and (d-f) their respective high-mag images.

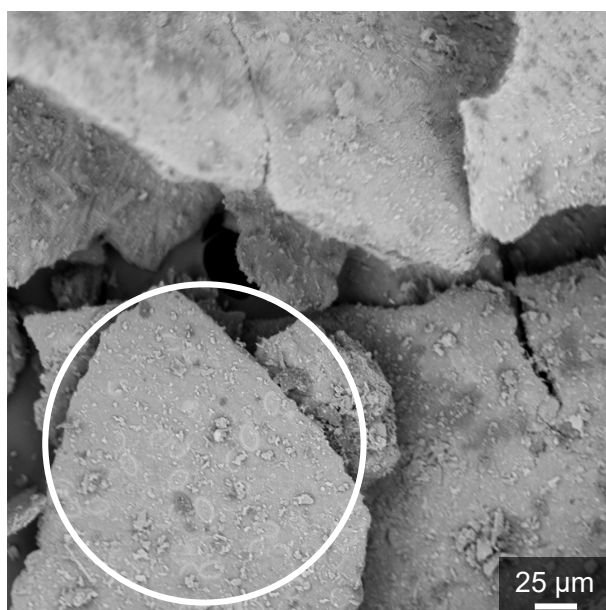


Figure 4.11: **Diatoms in *U. sp.*** SEM of *U. sp.* powder with several pennate-shaped diatoms circled in white.

ohnoi at 5 and 10 wt.%. SEM images reveal that the smallest pores typically correspond to tears within algal sheets, while larger pores arise from foam cell structures and collapsed

cellular domains.

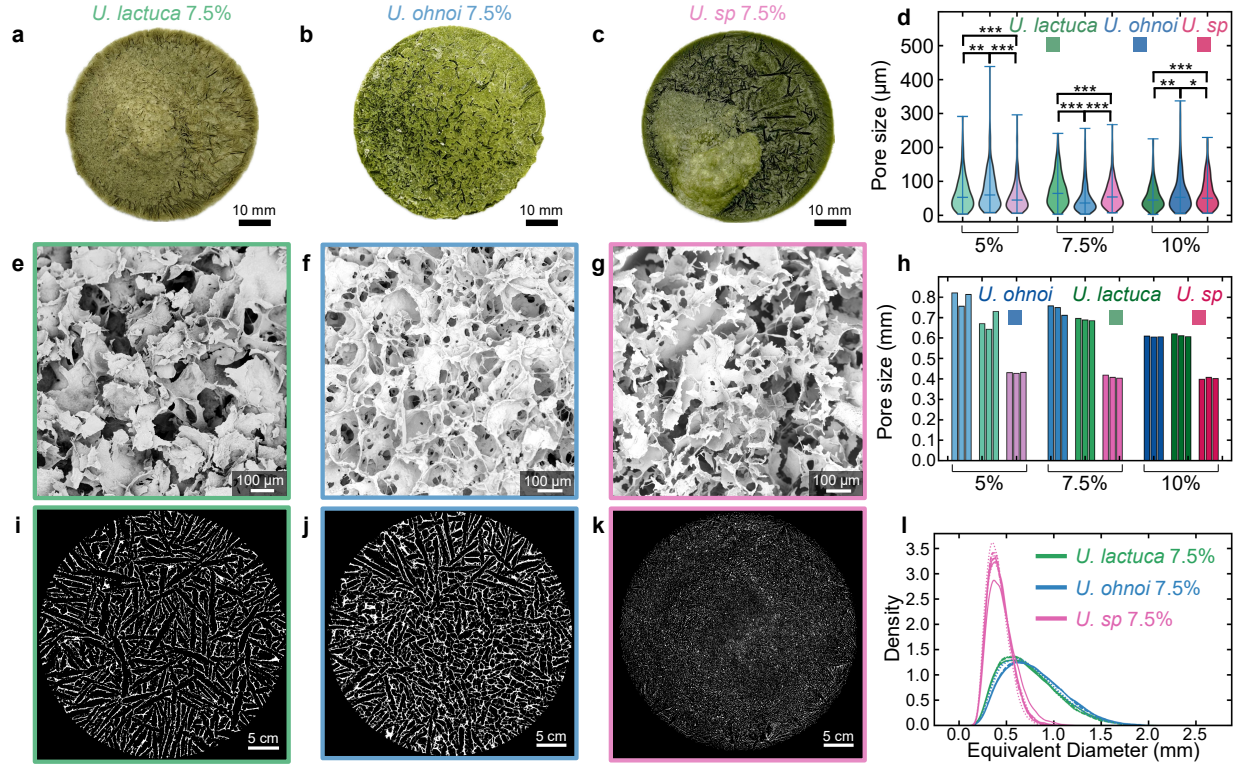


Figure 4.12: **Morphology from SEM and micro-CT of *Ulva* foams.** Micro-CT of **a** *Ulva lactuca*, **b** *Ulva ohnoi*, and **c** *Ulva sp.* at 7.5 wt.% concentrations. SEM of *Ulva* foams. SEM of **a** *Ulva lactuca*, **b** *Ulva ohnoi*, and **c** *Ulva sp.* at 7.5 wt.% concentrations. **g**, **h** Pore size distributions as measured from micro-CT **i** Pore size distributions measured from SEM.

To probe morphology at larger length scales, micro-CT was used (resolution: 34.4 μm), capturing macroporous structure complementary to SEM. Clear species-dependent differences emerge (Figure 4.12i-k). *U. lactuca* and *U. ohnoi* show distinct, elongated, and randomly oriented pore structures characteristic of ice templating, whereas *U. sp.* lacks this morphology and instead shows a more isotropic, fine-pore structure. Additionally, in *U. ohnoi* 10% and especially 7.5%, the interconnected web-like morphology observed in SEM manifests at larger scales as networks of smaller, interconnected pores extending over millimeter length scales and linking larger elongated voids. This hierarchical connectivity is also evident in *U. lactuca* but absent in *U. sp.*

Pore size analysis, as described in Section 4.1.7, shows that the equivalent diameter of the pores change with concentration in different ways for each species. *U. ohnoi* equivalent pore diameters decrease from 0.79 mm to 0.74 mm to 0.61 mm as concentration increases,

which is a 7.0% decrease from 5 wt.% to 7.5 wt.% and a 17.5% decrease from 7.5 wt.% to 10 wt.%. *U. lactuca*, in contrast had a 2.1% increase in equivalent pore diameter from 5 wt.% to 7.5 wt.%, going from 0.67 to 0.69 mm. The pore diameters then reduce by 11.1% between 7.5 wt.% and 10 wt.%, going from 0.69 mm to 0.61 mm. *U. sp* equivalent pore diameters decrease from 0.43 mm to 0.41 mm from 5 wt.% to 7.5 wt.%, a 4.9% decrease, and from 0.41 to 0.40 mm, a 2.3% decrease. Generally, all samples show a skewed-right distribution of equivalent pore diameters, with *U. ohnoi* and *U. lactuca* having broader distributions than *U. sp*. All species have reduced equivalent pore diameters at 10 wt.% compared to 5 wt.%, caused by their densification. However, the extent of these differences vary significantly. The 23.2% decrease for *U. ohnoi* is much larger than the 9.2% decrease for *U. lactuca*, which is larger again than the 7.0% decrease for *U. sp*. These trends are consistent with gelation-driven network densification in *U. ohnoi* (and to a lesser extent *U. lactuca*), whereas *U. sp*, which does not gel, shows limited structural evolution and lacks ice templated morphology.

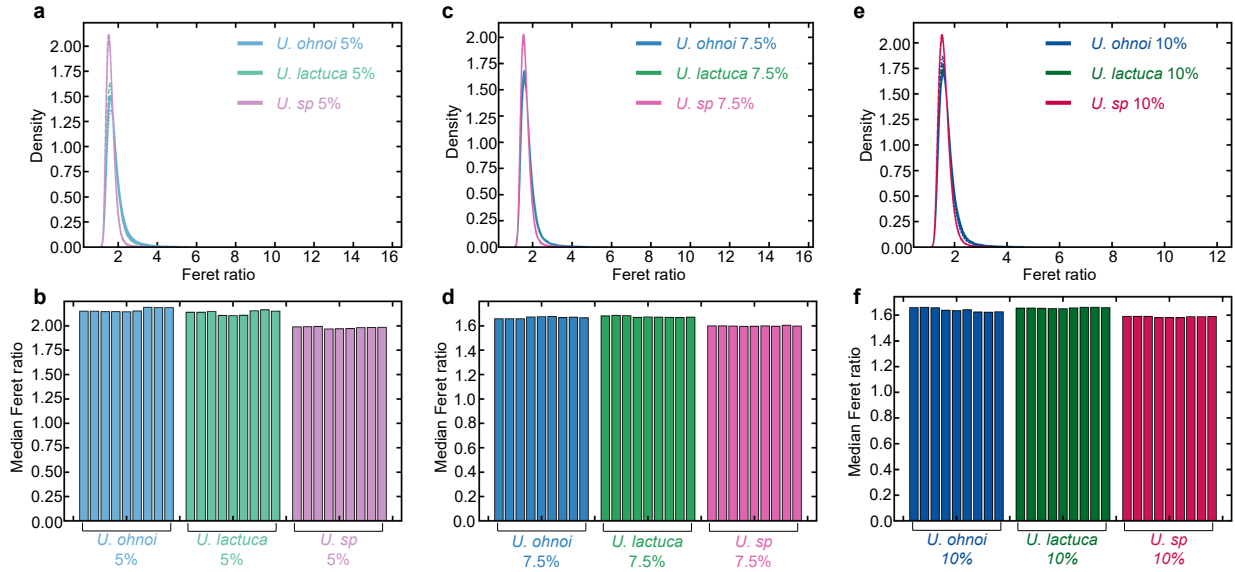


Figure 4.13: **Feret diameter ratios for *Ulva* acoustic foams** Feret ratio distributions and medians for *U. ohnoi*, *U. lactuca*, and *U. sp* for (a, b) 5 wt.%, (c, d) 7.5 wt.%, and (e, f) 10 wt.% concentrations.

Pore shapes are anisotropic across all species, with Feret ratios of 1.6–1.7, consistent with elongated pores formed via freeze-drying (Figure 4.13). *U. ohnoi* exhibits the largest change in pore anisotropy with concentration, indicating its pore shapes change the most

from low to high concentrations, while *U. sp* remains largely unchanged.

Porosities for *U. lactuca* and *U. ohnoi* match across concentrations, with porosities of 96.8%, 95.3%, and 93.7% for 5, 7.5, and 10 wt.% samples, respectively. *U. sp*, in stark contrast, has a much less obvious ice crystal morphology accompanied by reduced porosities. The 5, 7.5, and 10 wt.% samples have porosities of 95.9%, 94.2%, and 92.5%, respectively. This behavior is consistent with its lack of extended sheet structures and reduced ice-templated porosity.

Apparent densities of the foams range from 0.050 to 0.064 g/cm³ at 5 wt.%, 0.074 to 0.090 g/cm³ at 7.5 wt.%, and 0.100 to 0.116 g/cm³ at 10 wt.%. At all concentrations, *U. sp* has the highest apparent density and *U. lactuca* has the lowest.

Mechanical properties

Compression to 40% strain

Marked differences are seen in the stress-strain curves for each species, as shown in Figure 4.14a-c. For *U. ohnoi*, we consistently see a distinct peak at the transition between the linear elastic portion of the curve and the plateau region. In cellular solids, this is typically attributed to an initial failure of cell structure, often through buckling of the edge of a cell or rupture of the face of a cell. After the initial failure of the cell structure, the plateau region is where the foam's cells plastically deform at a relatively constant yield stress. At 10 wt.%, *U. ohnoi* has an erratic stress-strain curve typical of foams where the cells are failing (collapsing) in a brittle manner. Lower concentrations, however, show the expected relatively constant stress at the plateau region.

Compared to *U. ohnoi*, the other two *Ulva* species do not have peaks in yield strength before reaching the plateau region, and they do not show brittle failure behavior at any concentration. Additionally, both *U. sp* and *U. lactuca* show plateau regions that curve upwards very early in the loading curve. This indicates that *U. ohnoi* has a later onset of densification than the other two species, which would make it better for energy-absorbing applications.

These differences translate to distinct mechanical performance (Figure 4.14d-f). *U. ohnoi* has the highest strength at 40% strain at all concentrations compared to the other

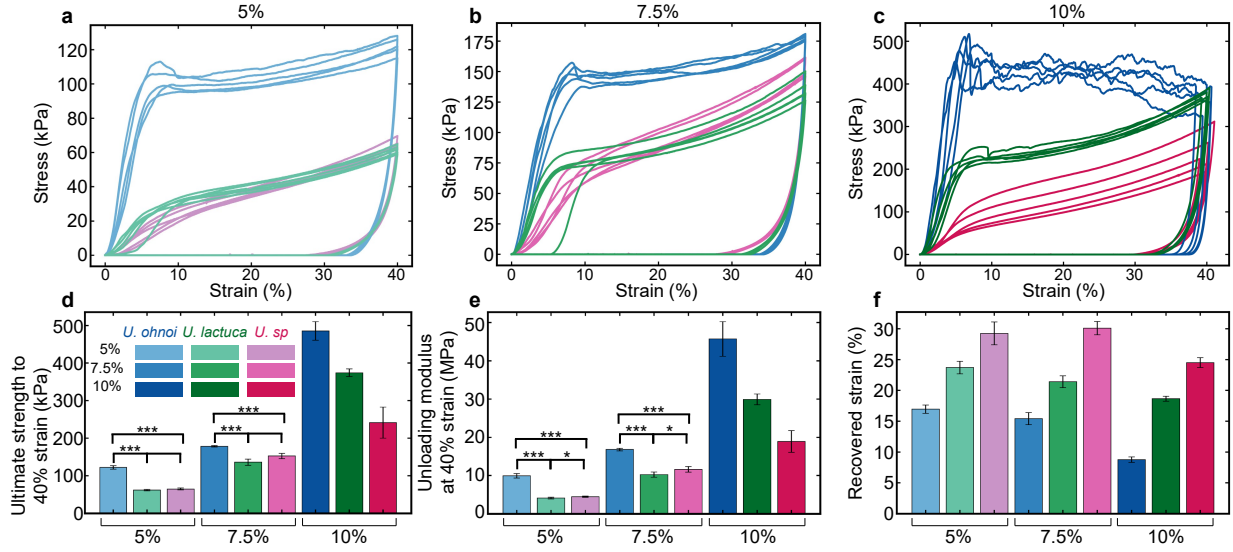


Figure 4.14: **Compression tests of *Ulva* foams.** Stress-strain curves of three *Ulva* species at (a) 5 wt.%, (b) 7.5 wt.%, and (c) 10 wt.% concentrations. Mean values for the (d) strength, (e) modulus and (f) recovery of *Ulva* foams from compression tests to 40% strain. The symbols *, **, and *** correspond to p-values of 10%, 5%, and 1%, respectively, from a Welch's t-test. In all other cases, there was no statistical evidence of a significant difference in means. The legend for all panels is in panel (d).

two species at the same concentrations. At 5%, *U. ohnoi* has an average strength at 40% strain of about 122 ± 5 kPa, which is 89% higher than the next strongest sample, *U. sp*, and 98% stronger than *U. lactuca*. At 7.5%, *U. ohnoi* has an average strength of 178 ± 2 kPa, which is only 17% stronger than the next strongest sample, *U. sp*, and 31% stronger than *U. lactuca*. At 10%, *U. ohnoi* has an average strength of 485 ± 25 kPa, which is 30% stronger than *U. lactuca* and 101% stronger than *U. sp*. *U. lactuca* has higher strength at 40% strain than *U. sp* at 10% concentration, but has slightly lower strengths at 40% strain at 7.5 wt.%. At 5 wt.%, *U. lactuca* and *U. sp* have no statistically provable difference in means. The same trends are seen for the unloading modulus. The ability for the foams to recover their height after compression to 40% follows the inverse trend of the unloading modulus. *U. ohnoi* recovers less strain at all concentrations than *U. lactuca*, which recovers less strain at all concentrations than *U. sp*. Additionally, for *U. ohnoi* and *U. lactuca*, the recovery decreases with increases in concentration. *U. sp* has approximately equal recovery at 5% and 7.5% concentrations, but lower recovery at 10%. For cellular solids, the properties of the sample are heavily affected by the relative

density of the samples. Relative densities range from 0.033 to 0.039 for the 5% samples, 0.055 to 0.059 for the 7.5% samples, and 0.074 to 0.076. Despite having comparable or lower relative densities, *U. ohnoi* exhibits the highest strength, stiffness, and extended plateau behavior. This indicates that its mechanical performance is not density-driven but instead reflects differences in internal structure and matrix connectivity, consistent with the web-like, gel-derived architectures observed in SEM and micro-CT.

4.2.3 Acoustic performance

Sound absorption and model fitting

The sound absorption performance of the algae-based porous samples (Table 4.1) was first evaluated experimentally using impedance tube measurements based on the transfer-function method, including all tested samples with multiple samples for each material type and concentration ratio (Figure 4.15a-c and Figure 4.16). Across all samples, a consistent broadband absorption behavior is observed. The absorption coefficient increases gradually from low frequencies, reaching peak values typically in the mid-frequency range (approximately 1000–2000 Hz), and remains relatively high at higher frequencies. Most samples achieve peak absorption values between 0.85 and 1.0, indicating strong sound attenuation performance within the measured frequency range.

At low frequencies (<500 Hz), all samples exhibit limited absorption, which is expected for porous materials with a thickness range of 21 to 26 mm. The transition from low to high absorption generally occurs between 500 Hz and 1500 Hz, depending on species. While the overall trends are consistent, noticeable variability is observed between samples, particularly for *U. lactuca* and *U. ohnoi*, suggesting sensitivity to internal structure, fabrication inconsistencies, and thickness differences. In contrast, *U. sp* samples show more stable and repeatable behavior across the frequency range.

As mentioned in Section 4.1.17, the JCA model was fitted using acoustic absorption curves derived from the average values of the experimentally measured acoustic absorption. Figures 4.15a-c show the predicted absorption coefficients of 5 wt.% samples at the average thickness, compared to the average absorption curves and the experimentally-measured

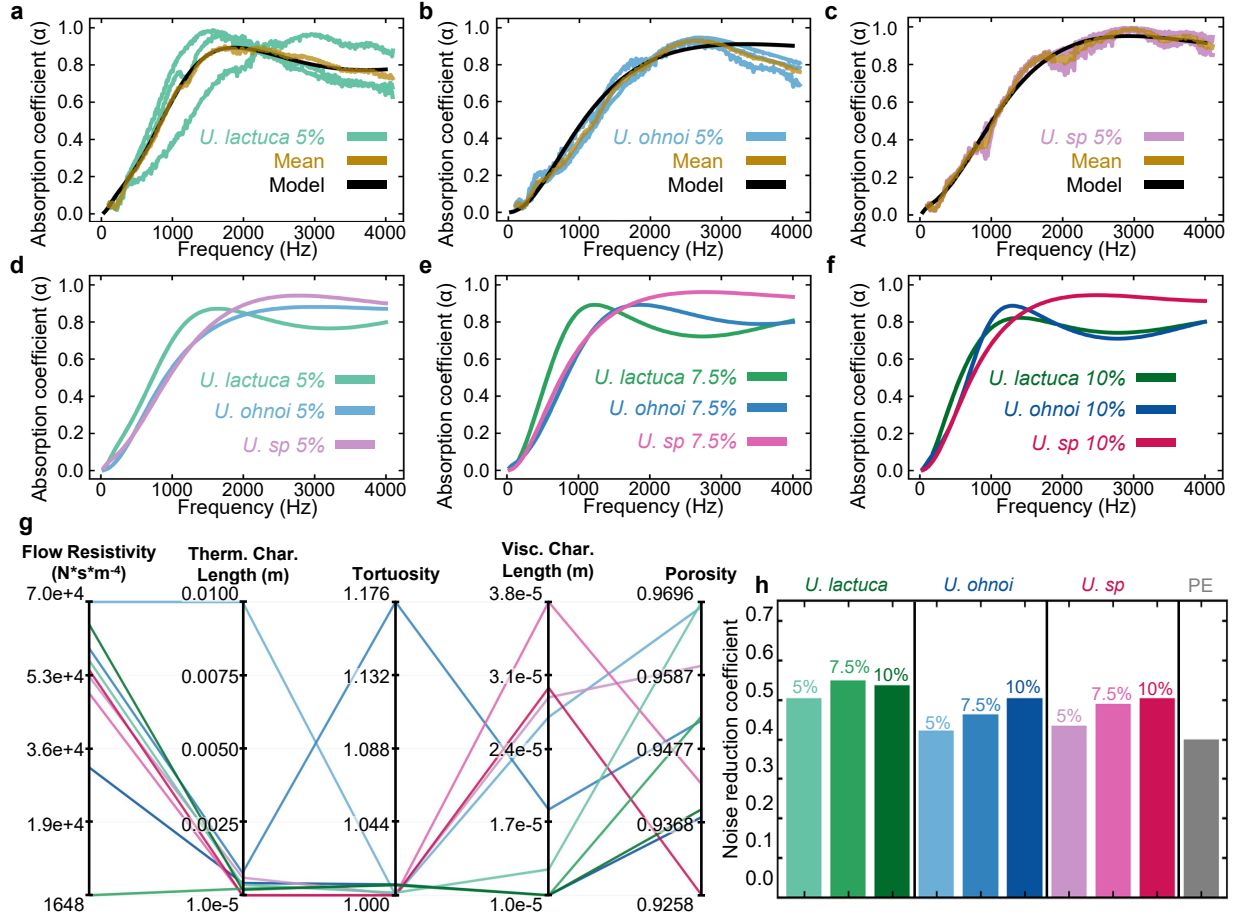


Figure 4.15: **Acoustic absorption of *Ulva* foams** Experimental acoustic absorption coefficients, the average absorption, and the JCA-predicted normal-incidence absorption coefficients at the samples' average thickness for (a) *U. lactuca*, (b) *U. ohnoi*, and (c) *U. sp* at 5 wt.%. Comparison of the JCA-predicted absorption coefficients at a uniform thickness of 25 mm for *Ulva* at (d) 5 wt.%, (e) 7.5 wt.%, (f) 10 wt.% concentrations and (g) their corresponding JCA material parameters. (h) Mean NRC for each species and concentration at 25 mm thickness, compared with a polyethylene (PE) commercial acoustic absorber.

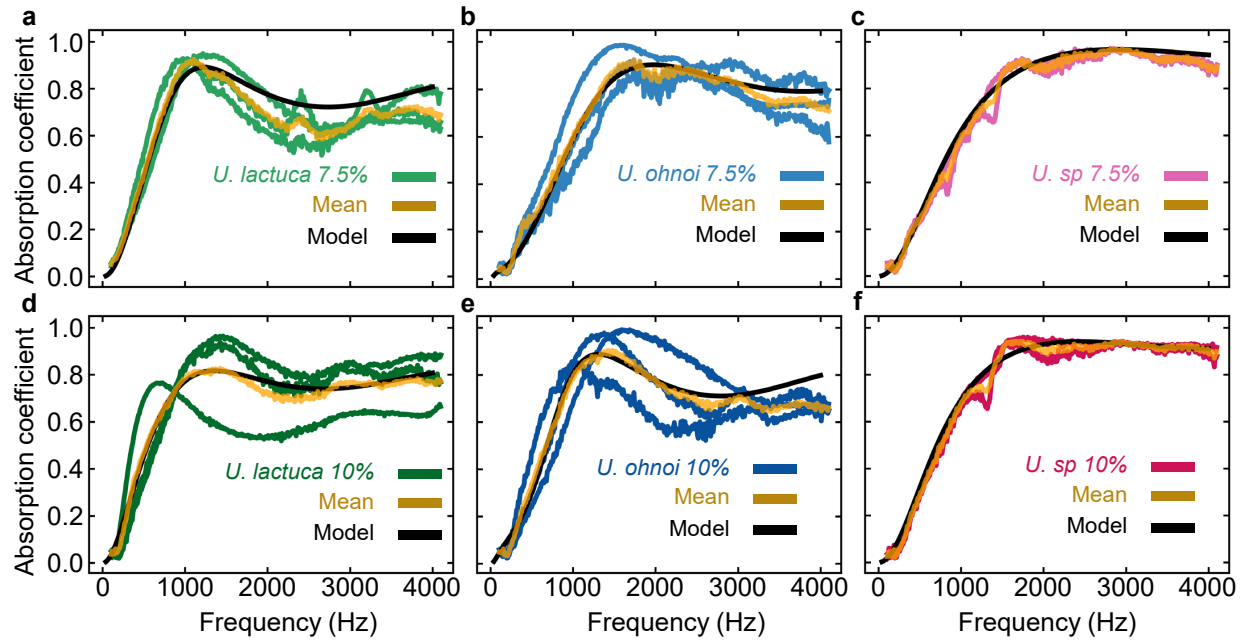


Figure 4.16: ***Ulva* JCA model fits** Measured, mean, and JCA-predicted absorption coefficients for *U. lactuca*, *U. ohnoi*, and *U. sp* samples at (a-c) 7.5 wt.% and (d-f) 10 wt.% concentrations, respectively. The mean curves in black represent the average acoustic absorption curve of all experimental samples. The model curves in orange represent the model fitted to the average acoustic absorption normalized to the average thickness of the samples.

absorption curves. These, as well as the corresponding curves for 7.5 wt.% and 10 wt.% samples (Figure 4.16) show the accurate representation of the experimental data with the predicted data from the model built off of the average of the experimental curves, capturing the general behavior and the characteristic rise in absorption within the frequency range. However, the fitting performance varies across samples, as reflected by the optimization error values obtained for each sample (Table 4.3).

For most of the samples, the low error values observed suggest a close match between model and experiment. In some samples, particularly the *U. lactuca* 7.5 wt.% and *U. ohnoi* 10 wt.% groups, lower R^2 values indicate that the JCA formulation captures the dominant acoustic behavior but does not fully represent local variations in the response. Higher error values are primarily associated with increased fluctuations in the measured data, especially at higher frequencies, which are not fully captured by the homogenized assumptions of the JCA model.

Species	Concentration (%)	Average thickness (mm)	Model fitting error (R^2 score)
<i>U. lactuca</i>	5	22.23	0.9933
<i>U. lactuca</i>	7.5	25.01	0.8563
<i>U. lactuca</i>	10	25.71	0.9755
<i>U. ohnoi</i>	5	21.95	0.9650
<i>U. ohnoi</i>	7.5	23.68	0.9815
<i>U. ohnoi</i>	10	24.85	0.9419
<i>U. sp</i>	5	23.77	0.9899
<i>U. sp</i>	7.5	23.87	0.9875
<i>U. sp</i>	10	25.50	0.9879

Table 4.3: Samples used in the JCA fitting process, organized by species, concentration, average thickness and model fitting error

	U. ohnoi		
Concentration	5%	7.5%	10%
Porosity	96.83 ± 0.09	95.34 ± 0.15	93.69 ± 0.10
Tortuosity	1.591 ± 0.123	1.392 ± 0.336	1.505 ± 0.020

Table 4.4: Porosity and tortuosity of U. ohnoi foam samples

	U. lactuca		
Concentration	5%	7.5%	10%
Porosity	96.82 ± 0.17	95.34 ± 0.16	93.70 ± 0.23
Tortuosity	1.614 ± 0.178	1.590 ± 0.040	1.532 ± 0.025

Table 4.5: Porosity and tortuosity of U. lactuca foam samples

	U. sp		
Concentration	5%	7.5%	10%
Porosity	95.91 ± 0.16	94.22 ± 0.07	92.51 ± 0.07
Tortuosity	1.593 ± 0.190	1.798 ± 0.195	1.559 ± 0.142

Table 4.6: Porosity and tortuosity of U. sp foam samples

Acoustic Comparison and Parameter Analysis

To enable a direct comparison of intrinsic material behavior, all samples were evaluated using the calibrated JCA model at a uniform thickness of 25 mm. Figures 4.15d-f compare the model curves of each species grouped by concentration. At 5 wt.%, all species show a gradual rise in absorption at low frequencies, reaching absorption coefficients in the range of 0.75–0.97 above approximately 2000 Hz. *U. ohnoi* and *U. sp* maintain absorption coefficients above 0.85 and 0.9, respectively, from 2000 Hz to 4000 Hz. *U. lactuca* displays a mid-range peak at approximately 1500 Hz with an absorption coefficient of approximately

0.9 before dropping to below 0.8 at 3000 Hz, then recovering slightly to 0.8 at 4000 Hz.

At 7.5 wt.%, variability between species increases noticeably. *U. sp* has a very similar curve at this concentration compared to 5 wt.%, but it maintains a slightly higher plateau with an absorption coefficient above 0.95 from 2000 Hz to 4000 Hz. *U. ohnoi* has a mid-range peak at approximately 1750 Hz with an absorption coefficient of 0.9 and gradually drops to 0.8 at 4000 Hz. *U. lactuca* has a more pronounced mid-range peak at 1200 Hz with an absorption coefficient of 0.9. Absorption then drops to about 0.7 at 2700 Hz before recovering up to 0.8 at 4000 Hz.

At 10 wt.%, *U. sp* displays approximately the same behavior as the 7.5 wt.% curve. *U. ohnoi* shows a much more distinct mid-range peak at 1250 Hz and an absorption coefficient of approximately 0.9. The absorption coefficient then drops to approximately 0.7 at 2700 Hz before recovering to 0.8 at 4000 Hz. *U. lactuca* shows very similar behavior, although with a slightly less pronounced peak with an absorption coefficient only slightly higher than 0.8.

Overall, increasing the density of *U. sp* samples does not significantly change the acoustic absorption behavior, demonstrating that for this species, the absorption coefficient is insensitive to the foam density. In contrast, *U. ohnoi* changes significantly from 5 wt.%, where it has acoustic absorption behavior similar to that of *U. sp* at 5 wt.%, to 7.5 wt.%, where its behavior is arguably in-between the behaviors of *U. sp* and *U. lactuca* at that same concentration, to 10 wt.%, where its behavior is almost the same as *U. lactuca* at that concentration. *U. lactuca* has a non-monotonic change in its acoustic absorption behavior, showing a more pronounced mid-range absorption peak and higher absorption at that peak at 7.5 wt.% than at lower or higher concentrations. Taken together, these results indicate that intrinsic material parameters, likely driven by material processing and composition, drive the acoustic behavior at a set thickness.

To investigate the influence of these material parameters on acoustic behavior, we compared the calibrated JCA parameters at 25 mm thickness using a parallel-coordinates plot (Figure 4.15g). Among the parameters, the viscous characteristic length (Λ) emerges as one of the most influential factors governing the shape and position of the absorption

peak. Samples with smaller viscous characteristic lengths, such as *U. ohnoi* at 5 wt.% and *U. lactuca* at all concentrations exhibit a noticeable shift in the absorption peak toward lower frequencies, creating the mid-range peak mentioned above. Samples with higher viscous characteristic lengths, such as all *U. sp* concentrations and *U. ohnoi* 5 wt.%, exhibit plateaus from 2000 Hz and higher, with no obvious absorption peaks. *U. ohnoi* 7.5 wt.% has a viscous characteristic length in-between these two sets of samples. Compared to the samples with viscous characteristic lengths lower than that of *U. ohnoi* 7.5 wt.%, it has an absorption peak at the highest frequencies and a broader peak than the other samples. Compared to the samples with higher viscous characteristic lengths, *U. ohnoi* 7.5 wt.% is the only one with an absorption peak at all, and the only one with a noticeable decrease in absorption after the peak. The sample with intermediate viscous characteristic length has intermediate acoustic absorption behavior. Viscous characteristic length directly correlates to the volume-to-surface area ratio of the pores. Higher volume-to-surface area ratios minimize reflections by reducing viscous resistance at the material surface. This allows high-frequency energy to penetrate deeper into the structure, leading to the more sustained absorption curves seen in the *U. sp* samples of all concentrations and *U. ohnoi* at 5 wt.%. Conversely, smaller volume-to-surface area ratios increase the effective density of the air inside the pores, lowering the speed of sound within the pores, shifting maximum absorption to lower frequencies. Additionally, smaller volume-to-surface area ratios increase viscous interactions, providing stronger but more localized absorption peaks at lower frequencies.

Other parameters do not exhibit significant effects on the acoustic absorption curves due to either negligible differences between samples (tortuosity and porosity) or due to the myriad of factors that can affect those parameters (flow resistivity). For example, flow resistivity is dependent on the porosity of the material, velocity of the air moving through the pores, and the pressure gradient within the pores. Morphology of the pores, including gaps, inlets/outlets, and imperfections on the pore surface, can affect both the velocity and pressure gradient within pores, making it difficult to derive physical material characteristics from differences in the flow resistivity^{268–271}. The data discussed in this section highlights

the dominant role of viscous characteristic length in tuning acoustic performance.

To provide a practical evaluation of the acoustic performance, the NRC was calculated for all predicted acoustic absorption curves (Figure 4.15h). Across all material types, the algae-based samples achieve NRC values in the range of approximately 0.40 to 0.55. Specifically, *U. lactuca* tends to outperform other samples at all concentrations, while *U. sp* and *U. ohnoi* only have NRCs at around 0.5 for 10 wt.% samples. In comparison, the commercial reference material exhibits an NRC of approximately 0.40. These results indicate that several of the developed bio-based absorbers not only match but actually exceed the performance of the commercial product at the same thickness. This is particularly significant given the relatively small thickness (25 mm), where achieving high absorption at mid-frequencies is typically challenging.

Overall, this data indicates that, independent of thickness, achieving high acoustic performance in algae-based, freeze-dried foams requires a balance between various pore-scale dissipation mechanisms. In particular, the viscous characteristic length plays a critical role in positioning the absorption peak in the mid-frequency range, directly impacting the NRC metric. Modifying pore morphology, size, and connectivity are all potential ways to tune these materials for desired acoustic performance.

For optimal sound absorption at a given frequency f , pore sizes should be similar to the thickness of the viscous boundary layer that is formed around the pore walls, given by²⁷²:

$$\delta_v = \sqrt{\frac{2\eta}{\rho_0\omega}} \quad (4.12)$$

where δ_v is the thickness of the viscous boundary layer. A comparison of the thickness of the viscous boundary layer in the frequencies of interests, to the foam's pore sizes is conducted using the SEM measurements, as described in section 4.2.2 and Figure 4.12. Figure 4.17 shows differences among species and concentrations.

For the 5% samples, all three species show relatively broad pore-size distributions, with dominant pore diameters mainly within the range of approximately 30–75 μm . *U. sp* 5% appears to have a slightly stronger contribution from smaller pores, while *U. ohnoi* 5% and

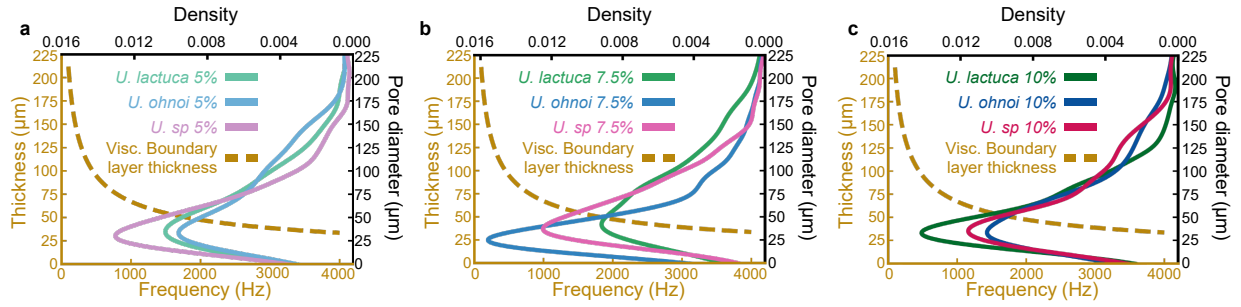


Figure 4.17: **Pore size distributions compared to viscous effects on acoustic absorption.** Comparisons of the pore distribution densities measured from SEM images to the viscous boundary layer thickness of most efficient absorption at each frequency. The viscous boundary layer thickness corresponds to the Frequency x-axis while the pore diameter kernel density estimates correspond to the Density x-axis at the top of the plots. Plots are shown for each species at (a) 5 wt.%, (b) 7.5 wt.%, and c, 10 wt.%.

U. lactuca 5% extend more clearly toward larger pore diameters. The broader distribution of the *U. ohnoi* and *U. lactuca* foams suggests that viscous losses may occur over a wider frequency range rather than being concentrated at a single frequency region. For the 7.5% samples, the pore-size distributions show stronger differences among the species. *U. lactuca* 7.5% closely intersects with the viscous boundary layer thickness curve. This indicates that, at that frequency range, the viscous boundary layer thickness becomes comparable to the dominant pore diameter of the sample, allowing stronger viscous interaction between the oscillating air and the pore walls. This is consistent with the absorption coefficient curve in Figure 4.15e, where *U. lactuca* 7.5% shows a clear absorption peak at a lower frequency compared with the other 7.5% samples. For the 10% samples, the pore-size distributions become more similar across the three species. Most of the dominant pore diameters are again concentrated around approximately 25–50 μm , although *U. lactuca* 10% shows a stronger contribution from smaller pores, while *U. sp* 10% and *U. ohnoi* 10% extend slightly more toward larger pore diameters. This suggests that increasing the concentration may produce a more consistent pore-size range across species, while still preserving some species-dependent differences in pore morphology. However, the pore size alone does not fully explain the behavior of each sample, as differences in pore connectivity, tortuosity, and flow resistivity likely affect how easily sound enters the material and how effectively acoustic energy is dissipated.

4.2.4 Flammability, additional mechanical testing, and biodegradation

One limitation of conventional acoustic absorption panels is the flammability of polymeric foams. Materials such as polyurethane typically rely on flame-retardant additives, which can increase smoke production and toxicity during combustion. In contrast, the intrinsic flammability behavior of biomatter-derived foams remains less explored. Here, we evaluate the flammability of foams derived from three *Ulva* species. In addition, to assess durability under practical use conditions, we examine strain-rate dependence and cyclic mechanical response. Given its intermediate composition and mechanical behavior, *U. lactuca* is selected as a representative system for these additional tests. Finally, we show the biodegradation profile of *U. lactuca* samples in seawater and discuss that while these samples readily degrade in seawater environments, there are complications in analyzing degradation profiles of organic materials.

Flammability

As previously mentioned, one of the issues with polymer foams, especially with those used in the built environment, is with the flammability of the polymers. Historical uses of algae as insulation were inadequate as flammability standards modernized. For modern polymer foams, anti-flammable additives may be used to make them up to code for buildings. Alternatively, polymer foams may have melting mechanisms that quickly take them away from the flame source upon contact with the flame.

In this study, flammability testing was carried out via a modified UL94 horizontal burning test, as mentioned in Section 4.1.15. In these tests, the PolySorb melted away from the flame immediately (Figure 4.18a-c), causing no further burning or melting of the material for the majority of the test. However, molten droplets persisted and for one sample, continued burning when retained on the support, highlighting the hazard associated with melt-dripping behavior.

In contrast, *U. lactuca* (Figure 4.18g, h) and *U. ohnoi* self-extinguish immediately upon removal from the flame, with no sustained combustion. Typically, phosphorous plays an

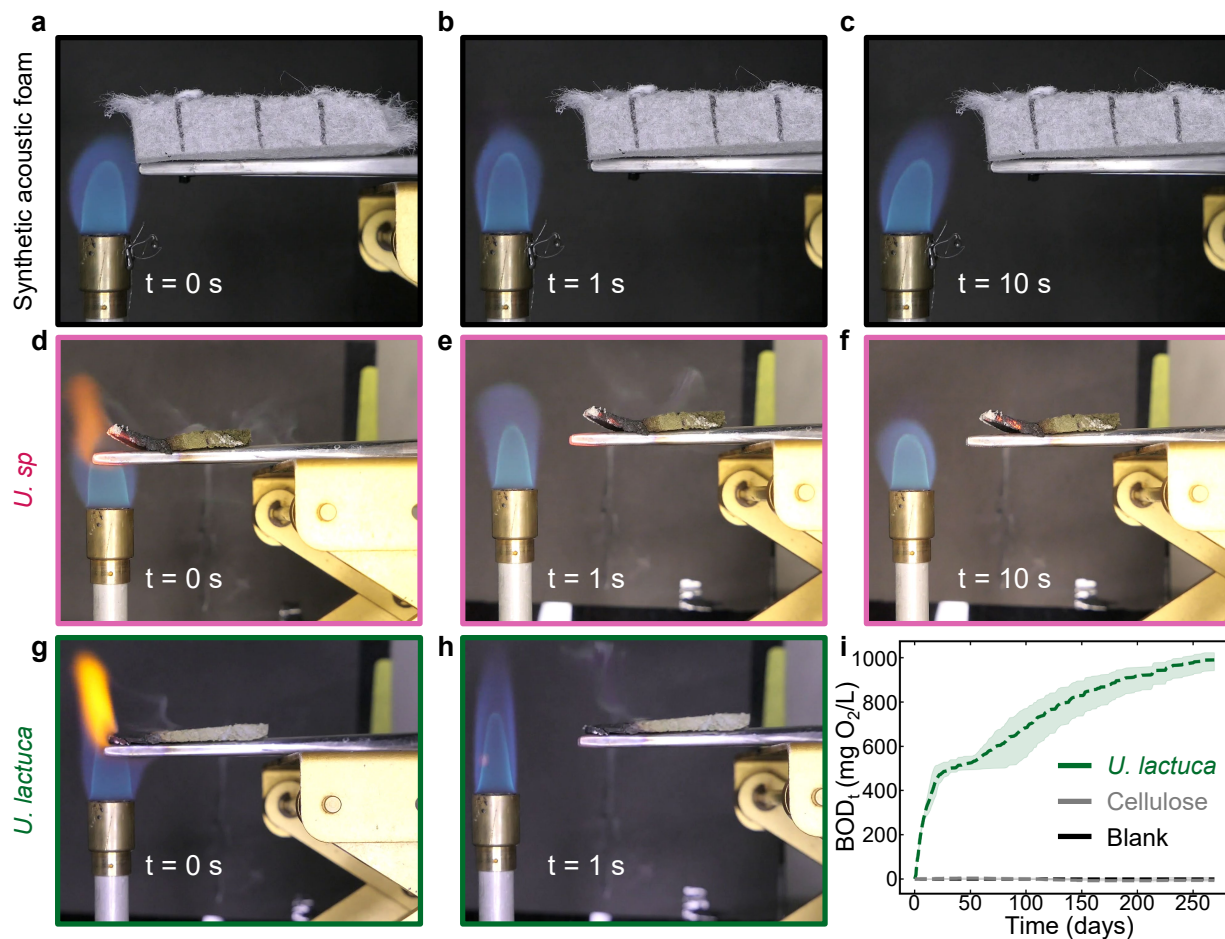


Figure 4.18: **Flammability and biodegradation.** Flammability testing of polymer foam **a-c**, *U. sp* **d-f**, and *U. lactuca* **g-h** just before removal from flame (left), just after removal from flame (right), and 10 seconds after removal from flame (right). Biochemical Oxygen Demand (BOD) curves of *U. lactuca* foam, a blank test vial, and a vial with microcrystalline cellulose **i**.

important role in anti-flammability properties of materials, especially when in conjunction with proteins for bio-based materials. For these samples, however, phosphorous contents are below 1% and in fact non-existent for *U. ohnoi* in particular, so it cannot be contributing to the anti-flammability nature of these materials. Instead, this ability is likely due to the high ash content of these two species of algae (25.6% and 39.6%, respectively). *U. sp* (Figure 4.18d-f) had inconsistent flammability behavior, where one sample self-extinguished immediately like the other two species, but other samples burned longer, with one sample even having an ember that lasted beyond the runtime of the test. *U. sp* has a lower ash content than the other two species (18.2%), so it may be that the approximately 7.4% difference in ash content between the *U. sp* and *U. lactuca* is causing the difference between

flammable and self-extinguishing. However, it is also important to remember that, per the discussion in Section 4.2.1, the composition of the ash for *U. sp* is significantly different from that of the *U. lactuca* and *U. ohnoi*, so it is likely that the composition of the different species contributes to the anti-flammability as well.

Strain rate tests

Prior sections have shown that for mechanical, rheological, acoustic, and compositional properties, *U. lactuca* tends to have properties between those of the other two species, so it is used for the next sections as a representative algal foam.

To further understand the mechanical properties of the *Ulva* foams, we first explored how the *U. lactuca* foams respond to different strain rates (Figure 4.21a-c and Figure 4.19). At 5 wt.% concentrations, there is no statistical evidence of a significant difference in means between all strain rates when it comes to strength and modulus, but the stress-strain curves are erratic at the 0.1%/min and 1%/min strain rates (Figures 4.19a-c). This causes a wide distribution of recovery percentages for the 5 wt.% samples, but there is no evidence of a significant difference between the mean recoveries for any of the 5 wt.% samples compressed at different strain rates (Figures 4.21a).

At 7.5% concentrations, the samples tested at a strain rate of 10%/min are stronger by 100% and 140% than the samples tested at 0.1%/min and 1%/min, respectively. There are significant differences between the stress-strain curves at each strain rate for this concentration (Figures 4.19d-f). At the slowest strain rate, the stress-strain curves all have very different loading behavior, with some samples reaching their plateau at higher strains than others, and some samples reaching their maximum strength at strains before the max compression strain of 20%. At the medium strain rate of 1% per minute, the curves are more consistent, especially when they reach 20% strain. However, the maximum stress is reached after the linear region of the loading curve, and the plateau region decreases from that maximum stress to the 20% strain point. At the fastest strain, the stress-strain curves look like they did in the previously mentioned 40% compression tests, with a flat plateau region and very consistent stress-strain curves. The percent recovery increases by about 10% per order of magnitude increase in strain rate, although there is no statistical evidence

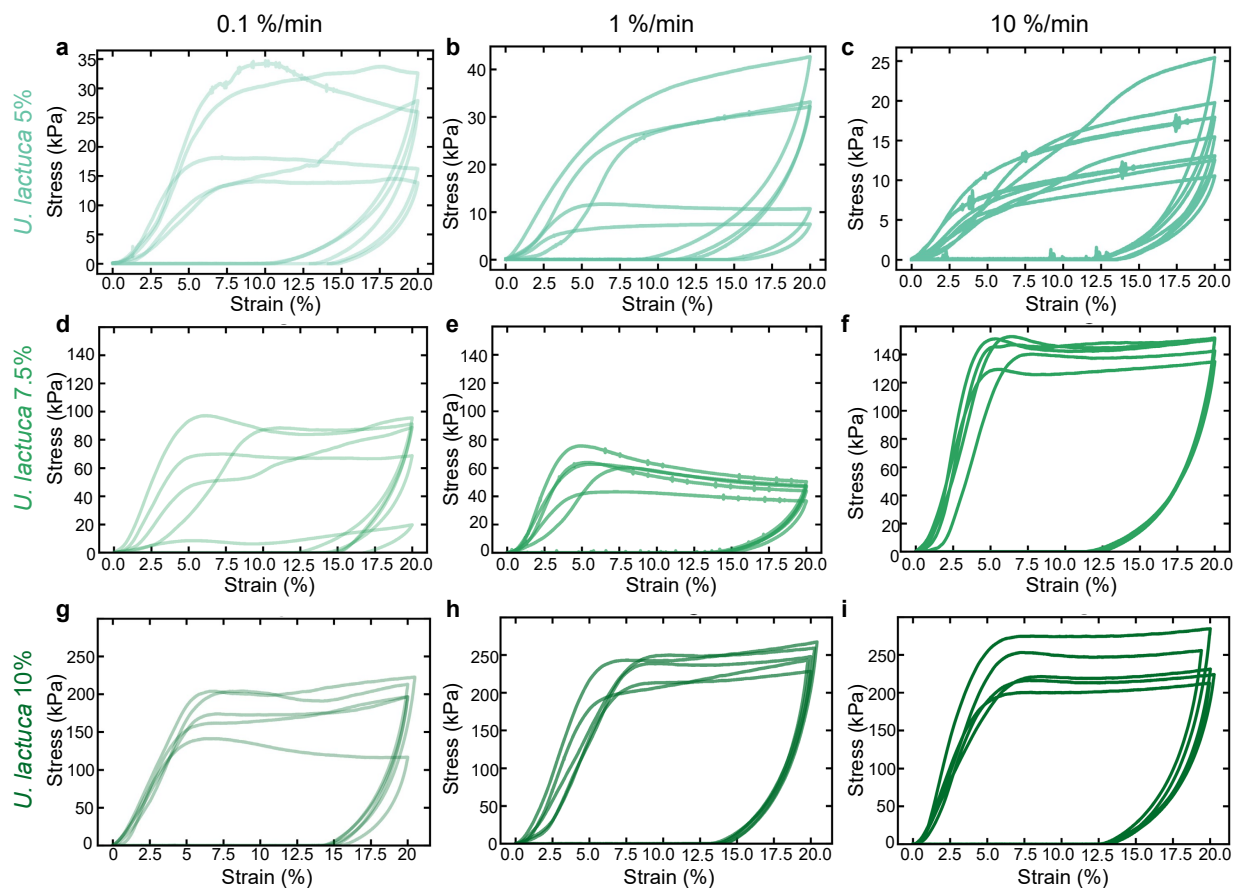


Figure 4.19: **Stress-strain curves for strain rate tests.** Stress-strain curves of compression tests up to 20% strain for *U. lactuca* (a-c) 5 wt.%, (d-f) 7.5 wt.%, and (g-i) 10 wt.% at strain rates of 0.1%/min, 1%/min, and 10%/min.

of a significant difference in means between the 0.1%/min and 1%/min samples.

At 10% concentrations, there is no statistical evidence of a significant difference in mean strengths and moduli for samples tested at medium and fast strain rates, and both are stronger than the samples tested at a slow strain rate by 28% and 25%, respectively. The stress-strain curves are relatively homogeneous at medium and fast strain rates, with more variation in shape at slow strain rates. Similarly to the 7.5% concentration samples, the recovery increases with each increase in strain rate, although to a lesser extent, with an approximately 5% increase in recovery percentage with each order of magnitude increase in strain rate.

Cyclic

The *Ulva* foams were then tested in cyclic stress-strain cycles up to 3% strain, which provided insights into how the foams dissipate energy over multiple load-unload cycles. First, we can see that the ratio of maximum stress in each cycle to the maximum stress in the first cycle decreases with each cycle for all concentrations.

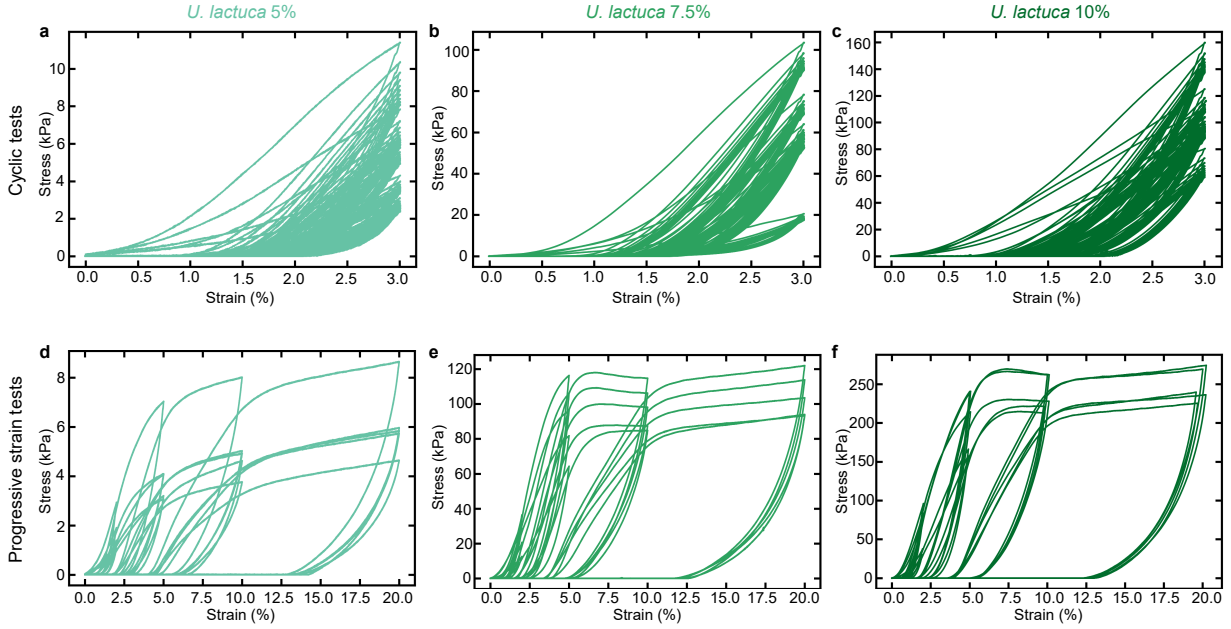


Figure 4.20: **Stress-strain curves for cyclic tests.** Stress-strain curves of (a-c) cyclic tests to 3% strain and (d-f) progressive-strain cyclic tests to 20% strain for *U. lactuca* at 5, 7.5, and 10 wt.%, respectively.

However, an interesting trend arises where the 7.5% samples have the lowest relative drop in max stress, followed by the 10% samples, then the 5% samples. We see a similar trend for the dissipated energy modulus ratios (need a better naming here). This was measured by taking the area under the curve for a loading cycle subtracted by the area under the curve for its corresponding unloading curve, and then dividing by that same area between curves for the first cycle of the test. We again see that the 7.5% samples dissipate the most energy, followed by the 10% samples, and then the 5% samples.

When work is done on a sample, such as by compression in this case, that energy can either be elastically stored or dissipated, typically in the form of friction. Samples that dissipate more energy can be expected to have more permanent deformation than samples that dissipate less energy. However, here we see the opposite trend where in both the strain

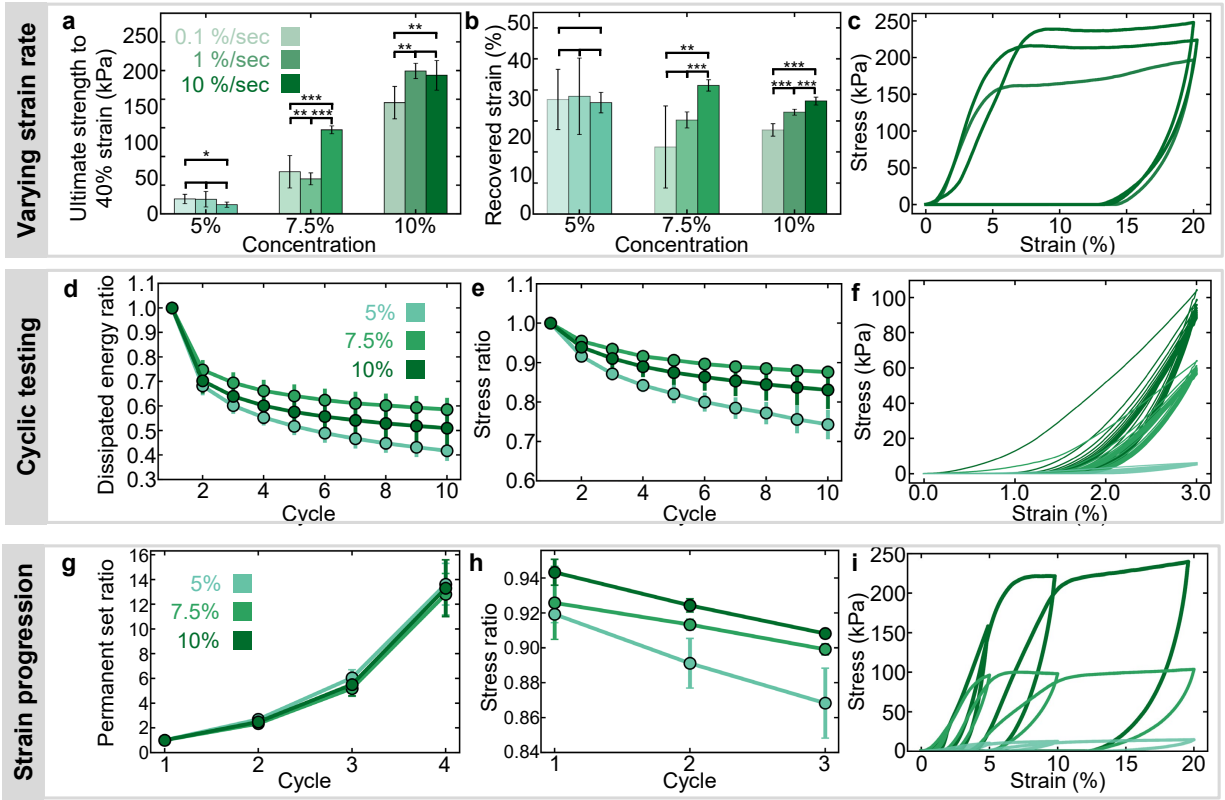


Figure 4.21: **Strain rate and cyclic compression tests.** (a) Strength, (b) recovery, and (c) representative stress-strain curves from compression tests to 20% strain with strain rates of 0.01%/min, 1%/min, and 10%/min. The symbols *, **, and *** correspond to p-values of 10%, 5%, and 1% or less, respectively, from a Welch's t-test. In all other cases, there was no statistical evidence of a significant difference in means. (d) Ratio of dissipated energy modulus, (e) strength ratio, and (f) representative stress-strain curves for compression tests to 3% strain for 10 load/unload cycles. (g) Permanent set, (h) stress ratio, and (i) stress-strain curves, for progressive-strain cyclic tests.

rate tests and the 40% compression tests, we see more recoverability for the 7.5% samples than the 10% samples, even though they also dissipate more energy. This decoupling of dissipation and permanent deformation suggests a distinct deformation mechanism, likely associated with the more open, interconnected structures observed at this concentration in SEM (Figure 4.12e-g), which can accommodate deformation while maintaining structural integrity.

Progressive strain cyclic tests

To further examine the non-linear elastic behavior of these foams, we conducted cyclic tests with increasing strain each cycle, from 2% to 20% (Figure 4.21g-i and Figures 4.20d-f). The stress-strain curves outline the same shape as their continuous 40% compression

tests including the loading and structure collapse regions. *U. lactuca* 5% shows a smooth transition from the loading section to the plateau section, and the plateau section actually has an increasing slope. At higher concentrations, there is an indication of plastic collapse of the structure where the stress decreases slightly after the linear section. This matches what was observed in the 40% compression tests described above. The stress-strain curves also show classic Mullins behavior. This typically refers to the stress-softening effect seen in rubbers and elastomers, but it can be used to describe other soft materials and foams as well. Ideal Mullins behavior is when a material is loaded, unloaded, and then loaded to a higher stress than the first cycle. The stress will be lower on the second loading than on the first loading until it passes the previous maximum strain, after which point it will rejoin and continue the shape of a virgin sample. At lower concentrations, *U. lactuca* foams require more strain to rejoin the original stress-strain curve, as indicated by the large gaps between where one cycle's max stress is reached and the strain at which that same stress is reached on the next cycle. On the other hand, 10 wt.% samples exhibit minimal deviation between cycles. In all cases, the gap between loading curves increases with increasing maximum strain. Another way to characterize this behavior is by looking at the change in permanent set over each cycle. The permanent set is defined by the difference in strain between where the loading curve starts (zero percent strain) and where the unloading curve reaches zero stress. In Figure 4.21, we see that all concentrations have very similar permanent set ratios at each cycle. Additionally, the permanent set always increases with each cycle, indicating the accumulation of plastic microstructural damage during each cycle.

Biodegradability

To calculate the degradation percentage of materials using biochemical oxygen demand (BOD), it is usually subtracted by the BOD of a blank cell to adjust for oxygen demand of microbes without samples to feed on, then divided by the theoretical oxygen demand (ThOD) of the sample. The ThOD must be calculated based on the molecular composition of the material, which is typically a trivial exercise for plastics that are often tested in these respirometry methods. However, this is significantly more complicated for biological samples where the composition can only be estimated or partially known, like the algae

used in this study. The ThOD was calculated using two different methods. The first used compositional data from CHN and XRF measurements to get the concentrations of oxidizing atoms in the sample and the second used literature to get an estimate of the amount of different simple molecules in the sample and calculate the weighted average of the ThOD for those molecules. Specifics on these methods are outlined in Section 4.1.11. Using the compositional analysis, the ThOD is calculated as 859.022 mg/g, while using the molecular analysis method the ThOD is calculated as 954.29 mg/g. For both of these methods, the ThOD is underestimated from what it should be because the percent degradation is measured as 115% and 104% for the two methods, respectively. This illustrates the difficulty in characterizing the degradation of bioplastics made from unconventional methods, such as biomass without extraction, waste streams, or other complex compounds such as lignin or extracellular matrices. For this reason, we will look at the BOD directly.

Aerobic biodegradation of a 7.5 wt.% *U. lactuca* foam was measured in terms of biochemical oxygen demand (BOD), which represents the amount of oxygen required by aerobic bacteria to break down the material in the reaction chamber. In the plot of BOD vs time for a 275 day biodegradation test shown in Figure 4.18, we can see what appears to be three distinct stages of degradation. From day zero to day 20, the BOD is in what is often called the carbonaceous phase, where bacteria are in a period of greatest growth, and therefore the greatest rate of demand on oxygen²⁷³. This is when the easiest compounds to degrade are attacked by the bacteria in the inoculum. As the bacteria run out of easily digestible carbonaceous material, the rate of oxygen demand begins to slow. From day 20 to approximately day 55, and even up to day 80 for one of the samples, the BOD shows a plateau phase. Over time, bacteria grow that are able to digest the nitrogenous compounds, breaking ammonia down into nitrites and nitrates, which leads to a third phase that is slower than the carbonaceous phase^{273–275}. For samples where no nitrification occurs, the BOD would look more like one continuous curve, but the two separate growth phases in Figure 4.18 indicate that nitrification occurs for *U. lactuca* in seawater. The plateau phase is common especially for samples in marine environments, where growth of nitrifying microbes can be inhibited compared to environments such as

wastewater or sewage. The average carbonaceous BOD (cBOD) is 529.09 mg/g, measured as the BOD at the time the sample plateaus, which was between 50 to 80 days, depending on the sample. The average nitrogenous BOD (nBOD) is 512.62 mg/g, measured as the cBOD subtracted from the BOD at the end of the test. As is expected from previous literature, the nBOD is less than the cBOD, although it still accounts for approximately 48% of the oxygen demand^{274–276}. From CHN analysis, *U. lactuca* has about 2.82% nitrogen and 27.76% carbon, but due to inefficiencies in degradation (not all the carbon is degraded), and due to the nitrogenous degradation process requiring 1.7 to 4.6 times the amount of oxygen than the carbonaceous process does²⁷⁷ (stoichiometrically derived), the nitrogenous contribution to biodegradation is substantial. This is consistent with previous studies of algal nBOD in streams and effluents^{276,278}. Micro-crystalline cellulose (MCC) was used as a control sample for which Figure 4.18i shows the BOD at the same level as that of the blank sample. This indicates that the microbes present in this batch of seawater do not have the cellulase or β -glucosidase enzymes to break down the cellulosic fraction of *Ulva*, which would mean that the BOD curve presented here shows incomplete degradation²⁷⁹. In other environments, such as soil, the carbonaceous portion of biodegradation would likely be higher than what we have observed in seawater due to the presence of cellulose-digesting bacteria and fungi.

4.3. Conclusion

Conventional acoustic absorption panels rely on petroleum-derived foams that are flammable and not end-of-life compatible. Here, we demonstrate a solvent-free, extraction-free route to acoustic foams from whole *Ulva* biomass via freeze-drying, yielding structurally and functionally tunable materials. Across species, composition and processing govern rheology, morphology, and ultimately performance. *U. ohnoi* forms gel-derived, interconnected networks that translate into higher stiffness, strength, and extended plateau behavior under compression, consistent with its higher content of sulfated polysaccharides and associated ionic species that promote network formation. On the other hand *U. sp* produces less connected structures with greater strain recovery. These differences are reflected across length

scales, with SEM and micro-CT revealing distinct pore architectures and concentration-dependent densification behavior.

All foams exhibit strong acoustic absorption, with *U. sp* matching or exceeding a commercial polymer foam across the full frequency range, and *U. lactuca* and *U. ohnoi* performing particularly well at mid-range frequencies, below 2500 Hz. Model-based analysis using a JCA framework shows that acoustic performance is governed by intrinsic transport parameters, particularly viscous characteristic length and flow resistivity, rather than geometry alone. This enables normalization across thickness and highlights a pathway for tuning absorption behavior through microstructural control.

Importantly, *U. lactuca* and *U. ohnoi* are intrinsically self-extinguishing, in contrast to melt-dripping commercial polymer foams, due to their high inorganic content. Together, these results demonstrate that whole-biomass algal foams can achieve competitive mechanical, acoustic, and fire-resistant performance without additives or chemical modification. Combined with their biodegradability and carbon-sequestering feedstock, these materials offer a viable route toward sustainable acoustic absorbers with programmable structure–property relationships.

5. Summary and Future Work

This dissertation examines how the processing of whole biomatter induces changes on the properties of bioplastics. In this process, I address the challenges inherent to processing and characterizing complex systems that distinguish these bioplastics from more commonly studied bioplastics such as those made from specific molecules extracted from natural resources. I present the use of spirulina, cellulose, and *Ulva* to make stiff, bulk bioplastics and energy-absorbing and acoustic-absorbing foams that are competitive in many ways with their synthetic counterparts.

The first aim of this thesis presents a novel bioplastic made from whole, non-extracted spirulina cells. Through the simple application of heat and pressure on a mold filled with spirulina, I show that the morphology changes according to the hot-press parameters, leading to a cohesive bioplastic at higher temperatures and pressures and a packed-powder morphology at lower temperatures and pressures. These changes in morphology lead to different mechanical properties and are likely driven by protein conformation and hydrogen bonding changes. The bioplastics have flexural strengths of up to 26 MPa and compression strengths up to 76 MPa, making them competitive with commercial plastics. I demonstrate the machinability, recyclability, and biodegradability of the bioplastics, as well as the use of additional processing and biobased and biodegradable additives to modify the properties to improve flexural strength up to 57 MPa.

While spirulina was the algae of choice for this study, more recent work has shown that other species of algae can undergo similar transformations into bioplastics^{280,281}. A deeper understanding of the mechanisms involved with the conversion of algae cells/tissue to bioplastics would allow for tuning of material properties by choosing or mixing specific species, or even by genetically modifying algae or growing them in environments that

can produce desired compositional properties. Model systems and molecular dynamics simulations coupled with modeling and machine learning techniques are a promising path towards this level of understanding and control of this process^{280,282}.

It is a major hurdle for discoveries in a lab to be accepted into the market, often due to cost reasons. While understanding the costs associated with producing algal bioplastics is not necessarily within the purview of academia, there are also technical hurdles to making algal bioplastics ubiquitous as a replacement for synthetic polymers. One major hurdle is the susceptibility to humidity and water of these bioplastics. First, the impacts of humidity over time need to be properly documented and understood. Then, there are various methods of water-proofing the bioplastics that can be experimented on. For example, applying beeswax or oils may hydrophobize the surface enough to prevent intrusion of water into the sample. While simple mixing of fatty acids may lead to a phase separation during hot-pressing, with the fatty acids melting out of the bioplastic, the compounding of algae with waxes, lipids, and plasticizers in twin-screw extruders or homogenizers may allow for creating a stable, hydrophobic sample. Compounding and pelletizing algae/lipid composites will also improve processability, allowing the materials to flow in equipment such as injection molders. The resulting materials are likely to be brittle, however, without some combination of compatibilizer and plasticizer. While the brittle nature of the bioplastics is also a problem that will need to be solved for commercial use, improving processability will allow for more complex and useful parts to be made out algae, which seems for now to be best used in single-use applications. An approach to improving processability could be to use steam in the compression or injection molding process to allow for both high heat and moisture, which could allow for easier flow of the material. It may also be attractive commercially to study the hot-pressing self-bonding of food waste as a way to valorize materials that would otherwise be thrown out, such as coffee grounds. Finally, it is important to properly understand how environmentally friendly the spirulina bioplastics actually are. LCAs are currently not standardized and published information is limited, making it difficult to compare the environmental effects of lab-scale processing of algal bioplastics with commercial-scale processing of polymers. To clearly make the argument

that these bioplastics are providing a quantifiable benefit to society, robust LCAs will need to be performed for these materials.

My second aim focused on understanding the effects of colloidal processing of bacterial cellulose and pectin on freeze-dried foams. The BC and pectin represented insoluble and soluble polysaccharides, respectively, allowing us to gain deeper insight into how different molecules might behave in algal systems. The BC was treated with CADES, which improved colloidal stability, promoted defibrillation of the BC fibers, and widened the foam pore network, increasing compressive strength at 40% by 25% and the loading stiffness by 146%. Rheology and SAXS revealed changes in the CADES-BC/pectin gel network, which directly mirrored changes in the mechanical properties. While this simple system was used to better understand algal mixtures in water, there is more work to be done on both improving the study as well as improving the foams themselves.

One aspect of cellular solids that was not explored in this study is how BC foam properties scale with relative density. To understand this, BC foams at different densities will need to be tested in compression, and their strength and unloading moduli can be plotted against a log-log plot versus relative density²⁸³. The relationship is expected to be linear, as with most cellular solids. However creating foams from different methods can lead to different pore structures that may lead to different relationships between mechanical properties and relative density. One example is to use directional freezing to create pores in specific directions, such as parallel to or orthogonal to the direction of compression. Another example may be to use additives to alter the ice crystal structure. Finally, optimizing the DES treatment to fully defibrillate and potentially even hydrolyze the BC into BC nanofibers or even nanocrystals would allow for a more stable, homogenous material and could significantly affect the network structure of BC foams. Comparing the scaling of mechanical properties versus relative density among different foam structures and using Gibson-Ashby models of cellular solids may help to further understand the impacts of processing on cellular materials made from biomatter.

Further understanding of the colloidal state is also needed for biomaterial processing going forward. The main unresolved issue with characterizing biomaterials is how SAXS

studies account for hierarchical materials. For example, SAXS was used in the second aim to better understand how BC fibers were entangled and the effects of pectin on those networks. While SAXS can tell one portion of the story, it does not capture the whole picture of BC fiber interactions due to the hierarchical nature of the BC fibers and therefore of their interactions. This will only get more complicated as more components are included, making it extremely difficult to use the same analysis methods on an algal colloid.

Finally, returning to the question of commercial viability, bio-based foams for applications such as packaging need to be quick and cheap to make, the way synthetic polymer foams currently are. While freeze drying is done at an industrial scale, the time required for drying will be more expensive than continuous processes. Therefore, alternate foaming methods must be explored. Synthetic polymers form membranes when heated past their glass transition temperature and foamed. BC does not do this, but other biopolymers such as xanthan gum and starch do, so it may be possible to use additives to continually process BC into foams. Additionally, it may be possible to foam a BC pellicle without having to blend it into a slurry first, keeping the inherent network of the BC pellicle intact.

Aim 3 used the understandings from the previous two aims to develop foams from whole algae tissues. Compositional differences between three *Ulva* species, *U. ohnoi*, *U. lactuca*, and an unknown species referred to as *U. sp*, were analyzed using TGA and XRF, showing that *U. sp* had a distinct lack of chloridic salts and lower sulfur content, but higher silicon content, and lower ash content overall. Additionally, FTIR indicated that *U. ohnoi* had more distinct peaks and higher intensities at wavenumbers corresponding to sulfides and polysaccharides, suggesting higher ulvan content. *U. sp* had a higher amount of silicates, making it difficult to interpret the main polysaccharide peaks. *U. lactuca* had higher lipids and -OH/N-H peaks, potentially indicating more lipid content and hydrogen bonding, which could be the result of higher polysaccharidic content or potentially effects from bound water. I then used rheology to better understand the gel behavior of the *Ulva* foams, showing that *U. ohnoi* and *U. lactuca* formed more extensible gels than *U. sp*, potentially due to the presence of salts in the two former species that can crosslink the ulvans present in the samples. Acoustic properties were then measured using impedance tube tests, showing

that the acoustic absorption capabilities of the algal foams rival or exceed the properties of a representative polyethylene commercial foam. The experimental data was fit to a JCA model to extract acoustic material parameters and normalize absorption coefficients by thickness. Generally, *U. lactuca* had excellent performance in mid-range frequencies (1000-2000 Hz), *U. sp* had excellent and consistent performance at higher frequencies (2000-4000 Hz), and *U. ohnoi* had performance similar to *U. lactuca* at low densities but similar to *U. sp* at high densities. This behavior was largely governed by the viscous characteristic length, which seemed to have the biggest effect on if samples had distinct mid-range peaks (*U. lactuca*), consistent high frequency absorption (*U. sp*), or somewhere in-between (*U. ohnoi*). Mechanical testing showed that the foams are both strong and stiff, while flammability and biodegradation testing provided evidence for the ability of the foams to replace current synthetic polymer foams with enhanced human and environmental safety as well as improved acoustic absorption.

Further exploration of the foam properties may be explored by enhancing the mechanical properties to more closely reflect the recoverability of synthetic polymer foams such as those made from polyurethane. For example, controlling the bound moisture in the samples will allow for control over the recoverability, and additives may provide a more robust structure. Directional freezing is again a promising method to improve both acoustic performance and mechanical performance, by aligning ice crystals in a way that is best suited for both. Additives would also likely affect the acoustic performance by modifying the parameters discussed previously. It would also be interesting to test if combining *U. lactuca* and *U. sp* slurries, whose foams had distinctly different absorption capabilities, would produce foams with combined absorption capabilities.

To that end, further improvement of the acoustic absorption can be developed using metamaterial design. The JCA models developed in this thesis can be used to develop a numerical model using FEA, to simulate acoustic behavior. This simulation can then be used to combine the foams with a Helmholtz resonator design to get both selective frequency absorption and broadband frequency absorption. Helmholtz resonator absorbers are cavities of air connected to the atmosphere via a smaller column of air. A classic

example of a Helmholtz resonator is a glass soda bottle, where the neck of the bottle is the column of air and the liquid-holding part of the volume is the air cavity. Helmholtz absorbers operate in the opposite way that Helmholtz resonators (like a soda bottle) do. Where resonators convert the motion of air into sound energy, absorbers convert sound energy into the motion of air, where the column of air in the neck acts like a spring to oscillate the air in the cavity and dissipate energy at the resonant frequency. While the absorbance is specific to this frequency, imperfect Helmholtz absorbers can reduce the absorbance at that specific frequency while broadening the absorption across a wider range of frequencies, and foam can be added to these absorbers to further broaden the range of frequencies absorbed. Helmholtz absorbers can be made out of algae using the techniques explored in aim 1, and *Ulva* foams can be added to the absorbers, which will create fully biodegradable and renewably sourced sound absorbers with both targeted and broadband frequency absorption.

6. List of Publications and Patents

6.1. Journal Publications

1. **Hareesh Iyer**, Mohammad Tabatabaei Manesh, Meghan Dillon, Ryan Kim, David Kim, Tomás Méndez Echenagucia, Eleftheria Roumeli. Biodegradable, self-extinguishing foams made from *Ulva* for broadband acoustic absorption. Under Review.
2. **Hareesh Iyer**, Aban Mandal, Michael Holden, and Eleftheria Roumeli. Modifying bacterial cellulose dispersions with deep eutectic solvent and pectin to tune the properties of open-celled foams. *RSC Applied Polymers*, 3(2):407-419. Publisher: The Royal Society of Chemistry.
3. **Hareesh Iyer**, Paul Grandgeorge, Andrew M. Jimenez, Ian R. Campbell, Malory Parker, Michael Holden, Mathangi Venkatesh, Marissa Nelsen, Bichlien Nguyen, Eleftheria Roumeli. Fabricating Strong and Stiff Bioplastics from Whole Spirulina Cells. *Advanced Functional Material*, 33(40):2302067. Publisher: Wiley-VCH
4. Aban Mandal, Kuotian Liao, **Hareesh Iyer**, Junhao Lin, Xinqi Li, Shuai Zhang, and Eleftheria Roumeli. Insights into controlling bacterial cellulose nanofiber film properties through balancing thermodynamic interactions and colloidal dynamics. *Molecular Systems Design & Engineering*, 9(10):1036-1050. Publisher: The Royal Society of Chemistry

5. Ian R. Campbell, Meng-Yen Lin, **Hareesh Iyer**, Mallory Parker, Jeremy L. Fredericks, Kuotian Liao, Andrew M. Jimenez, Paul Grandgeorge, Eleftheria Roumeli. Progress in Sustainable Polymers from Biological Matter. *Annual Review of Materials Research*, 53:81-104. Publisher: Annual Reviews
6. Jeremy L. Fredericks, **Hareesh Iyer**, Robin McDonald, Jeffrey Hsu, Andrew M. Jimenez, Eleftheria Roumeli. Spirulina-based composites for 3D-printing. *Journal of Polymer Science*, 59(22):2878-2894. Publisher: Wiley-VCH

6.2. Patents

1. Eleftheria Roumeli, Ian Campbell, Andrew M. Jimenez, Michael Holden, Paul Grandgeorge, Kuotian Liao, **Hareesh Iyer**. Bioplastic materials obtained from biological matter. Patent number: US20240076478A1

6.3. Conference Proceedings

1. **Hareesh Iyer**, Mohammad Tabatabaei Manesh, Meghan Dillon, David Kim, Ryan Kim, Tomás Méndez Echenagucia, Eleftheria Roumeli. Algal Biomatter Metamaterials for Acoustic Insulation Applications. *Materials Research Society* Seattle, WA, United States, 2025, April 7-11
2. **Hareesh Iyer**, Aban Mandal, Michael Holden, and Eleftheria Roumeli. Modifying Bacterial Cellulose Dispersions with Deep Eutectic Solvent and Pectin to Tune the Properties of Open-Celled Foams. *American Chemical Society* San Diego, CA, United States, 2025, March 22-27
3. **Hareesh Iyer**, Aban Mandal, Michael Holden, Eleftheria Roumeli. Effects of Chemical Treatments and Additives on Bacterial Cellulose Foams. *Materials Research Society* Seattle, WA, United States, 2024, April 22-26
4. Aban Mandal, Julia Didier, **Hareesh Iyer**, Ian Campbell, Kuotian Liao, Eleftheria Roumeli. Tailoring sustainable materials through biopolymer engineering: from sea-

- weed adhesives to bacterial cellulose networks. *American Chemical Society Spring Meeting 2025*, San Diego, CA, March 23-27, 2025
5. Aban Mandal, Kuotian Liao, **Hareesh Iyer**, Junhao Lin, Shuai Zhang, Xinqi Li, Eleftheria Roumeli. Solvent-Mediated Control of Bacterial Cellulose Nanofiber Conformations: From Colloidal Dynamics to Solid-Film Properties. *APS March Meeting 2025*. Anaheim, CA, March 16-21, 2025
 6. Kuotian Liao, Danli Luo, Yiyang Sun, Ian Campbell, **Hareesh Iyer**, Catherine Brinson, Cynthia Rudin, Kayla Sprenger, Linda Schadler, Nadya Peek, Eleftheria Roumeli. ML-Assisted Autonomous Assistant Robot for High-Throughput Algal Bioplastics Fabrication. *Materials Research Society Spring Meeting 2025*, Seattle, WA, April 7-11, 2025
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 8. Julia Amorim, Kuotian Liao, **Hareesh Iyer**, Eleftheria Roumeli. Exploring the Mesoscale Framework of Bacterial Cellulose: Insights into 3D Network Formation during Biosynthesis. *Society for Biomaterials Regional Symposia 2024*, Seattle, WA, September 19-20, 2024
 9. Julia Amorim, Kuotian Liao, **Hareesh Iyer**, Eleftheria Roumeli. Understanding 3D networks structure during biosynthesis of cellulose from bacteria. *ACS Colloid and Surface Science Symposium*, Seattle, WA, June 23-26, 2024
 10. Aban Mandal, **Hareesh Iyer**, Eleftheria Roumeli. Nanocellulose networks: Utilizing colloidal processing principles to tune the mechanical properties of solid foams. *Materials Research Society Spring Meeting 2024*, Seattle, WA, April 22-27, 2024
 11. Kuotian Liao, Mallory Parker, **Hareesh Iyer**, Aban Mandal, Taylor A Hilton, Rebekah I Brain, E. Roumeli. Bacterial Cellulose-based Inks: Enabling Mechanical

Property Control in Sustainable 3D Printing. *Materials Research Society Spring Meeting 2024*, Seattle, WA, April 22-27, 2024

12. Julia Amorim, Kuotian Liao, **Hareesh Iyer**, Eleftheria Roumeli. Understanding 3D networks structure during biosynthesis of cellulose from bacteria. *Materials Research Society Spring Meeting 2024*, Seattle, WA, April 22-27, 2024
13. **Hareesh Iyer**, Paul Grandgeorge, Ian Campbell, Mallory Parker, Eleftheria Roumeli. Using carbon sequestering microorganisms to form strong biodegradable plastics. *American Chemical Society Spring Meeting 2024*, New Orleans, LA, March 17-21, 2024
14. Ian Campbell, **Hareesh Iyer**, Paul Grandgeorge, Mallory Parker, Eleftheria Roumeli. Protein-rich photosynthetic biomass as a matrix for high strength and stiffness bioplastics. *American Chemical Society Spring Meeting 2024*, New Orleans, LA, March 17-21, 2024
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16. Paul Grandgeorge, Andrew M. Jimenez, Ian R. Campbell, **Hareesh Iyer**, Michael Holden, Eleftheria Roumeli. Fabricating Strong Bioplastics from Algal Biological Matter: Challenges and Opportunities. *Society of Engineering Science Annual Technical Meeting 2023*, Minneapolis, MN, 10/2023
17. Ian R. Campbell, **Hareesh Iyer**, Paul Grandgeorge, Andrew M. Jimenez, Michael Holden, Eleftheria Roumeli. Transformation Of Whole Biomatter To Strong And Stiff Bioplastics. *Plastics Recycling and Upcycling Gordon Research Conference* July 9-14, 2023, New Hampshire
18. Ian R. Campbell, **Hareesh Iyer**, Paul Grandgeorge, Andrew M. Jimenez, Michael Holden, Eleftheria Roumeli. From plant and algae cells to stiff and strong bioplastics. *2023 TAPPI Nanotechnology Conference*, June 12 - 16, 2023 Vancouver, BC

19. **Hareesh Iyer**, Ian R. Campbell, Michael Holden, Paul Grandgeorge, Andrew M. Jimenez, Eleftheria Roumeli, Strong biodegradable bioplastics from biological matter, *American Chemical Society Fall Meeting 2023*, San Francisco, CA, August 13-17, 2023
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26. Mengyen Lin, Ian R. Campbell, Kuotian Liao, **Hareesh Iyer**, Andrew Jimenez, Paul Grandgeorge, Eleftheria Roumeli, Utilizing unprocessed plant and algal mat-

- ter in structural materials and plastics. *Annual Green Chemistry & Engineering Conference*, Reston, VA, 06/2022
27. Ian R. Campbell, **Hareesh Iyer**, Michael Holden, Andrew Jimenez, Paul Grandgeorge, Eleftheria Roumeli, Thermomechanical processing of plasticized plant and algal matter. *Annual Green Chemistry & Engineering Conference*, Reston, VA, 06/2022
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