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Exploring Agar as a Sustainable Alternative to Petroleum-Based Binders

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16. Abstract Over 90% of paved roads in the United States are surfaced with asphaltic materials comprised of aggregate and a non-renewable petroleum-based asphalt binder. The sourcing of such a vital civil engineering material is both environmentally (i.e., contributing to pollution as well as greenhouse gas and volatile organic compound emissions) and economically problematic (i.e., contributing to volatile material costs and geopolitical tensions). Despite these drawbacks, the overall need for transportation infrastructure is only expected to increase, requiring the construction industry to shift to biobased material alternatives for petroleum-based construction materials. The construction industry has recognized this need and has begun investigating alternative materials for plastics, adhesives, and binders, like asphaltic materials. Notably, research regarding alternative asphalt binders has primarily focused on the use of bio-oils and less research has been aimed at biopolymers. To this end, this project aims to characterize and evaluate agar, a biopolymer extracted from red seaweed, as a direct replacement material for use in transportation infrastructure. This work is motivated by the promising photosynthetic traits of macroalgae (i.e., seaweed) and the synergistic features of high concentration gels and thermoplastic petroleum materials. In this work, high concentration (i.e., 5-10% w/w) agar gels were explored using traditional engineering testing techniques for pavements and standardized durability testing (Chapter 2). With the goal of improving moisture characteristics, the diffusion traits of both physically (i.e., polyurethane coated, inert filler materials) and chemically modified (i.e., maleic anhydride treated) agar-based materials were classified and modeled (Chapter 3). Lastly, agar-based materials were evaluated in composite materials using traditional dense-grade aggregate in order to gage suitability for pavement structures. The results of this project illustrate the need for additional research but highlight the potential for algae-derived materials like agar to be used in civil infrastructure applications.			
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Abstract

Over 90% of paved roads in the United States are surfaced with asphaltic materials comprised of aggregate and a non-renewable petroleum-based asphalt binder. The sourcing of such a vital civil engineering material is both environmentally (*i.e.*, contributing to pollution as well as greenhouse gas and volatile organic compound emissions) and economically problematic (*i.e.*, contributing to volatile material costs and geopolitical tensions). Despite these drawbacks, the overall need for transportation infrastructure is only expected to increase, requiring the construction industry to shift to biobased material alternatives for petroleum-based construction materials.

The construction industry has recognized this need and has begun investigating alternative materials for plastics, adhesives, and binders, like asphaltic materials. Notably, research regarding alternative asphalt binders has primarily focused on the use of bio-oils and less research has been aimed at biopolymers. To this end, this research project aims to characterize and evaluate agar, a biopolymer extracted from red seaweed, as a direct replacement material for use in transportation infrastructure. This work is motivated by the promising photosynthetic traits of macroalgae (*i.e.*, seaweed) and the synergistic features of high concentration gels and thermoplastic petroleum materials.

In this work, high concentration (*i.e.*, 5-10% w/w) agar gels were explored using traditional engineering testing techniques for pavements and standardized durability testing (Chapter 2). With the goal of improving moisture characteristics, the diffusion traits of both physically (*i.e.*, polyurethane coated, inert filler materials) and chemically modified (*i.e.*, maleic anhydride treated) agar-based materials were classified and modeled (Chapter 3). Lastly, agar-based materials were evaluated in composite materials using traditional dense-grade aggregate in order to gauge suitability for pavement structures. The results of this research illustrate the need for additional research but highlight the potential for algae-derived materials like agar to be used in civil infrastructure applications.

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1. Introduction

1.1 Motivation

Economic and Environmental Impacts of Petroleum Dependence in Construction

Within the built environment, petroleum is a primary material source for a vast spectrum of materials. Through the process of refining, petroleum is distilled to remove high value fractions like gasoline, kerosene, and diesel and the remaining residual material fractions can be further distilled to obtain hydrocarbon rich feedstocks for the production of synthetic polymers (*e.g.*, ethylene, propylene) (Engelsmann et al. 2012; Geyer et al. 2017) or high boiling point residues (*e.g.*, asphaltic binders and bitumen). These fractions of crude oil were once considered low value but have quickly become incredibly important to the construction industry (Little et al. 2018).

Construction materials derived from petroleum include plastics (*e.g.*, polyvinyl chloride, polyethylene, polypropylene, polystyrene) (Billington et al. 2014; Siracusa and Blanco 2020), adhesives (*e.g.*, polyurethane, resins, epoxies, silicones) (Magalhães et al. 2019), and binders (*e.g.*, asphalt for roadways, roofing, coatings, and waterproofing). The versatility of petroleum-based materials has led to their inclusion in piping, insulation, waterproofing membranes (Agarwal and Gupta 2017; Mohan et al. 2021; Nanda et al. 2022) and composite materials for both nonstructural and structural components (Islam et al. 2022; Nwankwo et al. 2023). Petroleum-based materials are widespread in all civil engineering sectors, comprising utilities, building components, and the vast majority of paved roadways.

The sourcing and widespread use of non-renewable petroleum-based materials is problematic in multiple facets. The accelerated depletion of non-renewable crude oil has led to limited material availability, with literature estimating that current petroleum reserves will only last for approximately 46 more years (Penki and Rout 2021). Despite the depletion of natural reserves, the global demand of petroleum products rose by 1.3% in recent years (*Global Energy and CO2 Status Report* 2018). In turn, petroleum-based materials illustrate volatile costs and are highly dependent on geopolitical dynamics.

In addition to volatile material costs, the sourcing of petroleum-derived materials is environmentally problematic in multiple facets. Crude oil exploration and drilling disrupts natural animal habitats and pollutes natural water, land, and air resources (Eneh 2011). The processing of petroleum to create petrochemical products is energy intensive, emits CO₂ and unburned

hydrocarbons, and contributes to air pollution (Thives and Ghisi 2017). Further, the relatively short lifespan of petroleum-based materials coupled with their prolonged decomposition time and the fact that they are often disposed of improperly, means many of these materials linger in the environment or accumulate in the ocean and on land (Oberti and Paciello 2022).

By virtue of its size, the construction sector represents an enormous material demand, consuming up to 40% of global raw materials annually (Malabi Eberhardt et al. 2021). This material consumption and global demand for durable and resilient buildings and transportation networks is only expected to increase with urbanization and population growth (Sun et al. 2022). The widespread use of petroleum-based materials in the construction industry illustrates an effective entry point for improving global industrial sustainability: To meet the global environmental and economic need for sustainable construction materials, additional direct alternatives for petroleum-based construction materials need to be investigated.

The Growing Need for Sustainable and Resilient Transportation Infrastructure

One subsector of the construction industry that largely relies on petroleum-based construction materials is transportation infrastructure. Over 90% of paved roads in the United States are surfaced with asphaltic materials comprised of aggregate and a non-renewable petroleum-based binder (Asphalt Paving Association of Iowa 2020). The vast majority of commercial petroleum-based asphalt binders are produced by continually distilling crude oil until a thick residue is obtained. Although asphalt binders only comprise approximately 9-18% of flexible pavement volume (Little et al. 2018), they are the most expensive and economically variable material in asphalt mixtures (*Reclaimed Asphalt Pavement in Asphalt Mixtures: State of the Practice* 2011).

Asphalt materials are most commonly constructed with Hot Mix Asphalt (HMA) created by mixing heated aggregate and petroleum-based binder at temperatures higher than 150 °C (300 °F) (Rubio et al. 2013). Once heated and mixed, HMA is transported directly to site and discharged into the hopper of a paving machine. The paver spreads the mix over the pavement surface and a roller compacts the mixture to a required pavement density (Little et al. 2018). This process is often repeated to create layers of compacted HMA. The construction of asphalt requires high working temperatures and diesel machinery, which requires substantial manufacturing energy. Further, the production of materials and construction methods used in asphalt pavement applications emits greenhouse gas emissions and toxic volatile organic compounds that pose health risks to exposed construction workers (Cui et al. 2020).

The construction of transportation network infrastructure (*e.g.*, roads and highways) is known to represent a significant portion of the direct and indirect negative environmental consequences of construction and statistics indicate that vehicle miles traveled in the U.S. are expected increase at an average rate of 1.0% annually over the next 20 years (Federal Highway Administration 2021). The increasing demand of transportation infrastructure necessitates improved sustainability *and* durability of pavement structures in order to minimize maintenance, rehabilitation, and environmental costs of future construction.

1.2 State of the Art of Pavements

The Functional Requirements of Pavements

Asphalt pavement structures are typically designed with consideration of four layers, as shown in Figure 1.1. From the top to the bottom of the pavement structure, these layers are designated as the surface (or wearing) course (*i.e.*, asphalt comprised of aggregate and a petroleum-based binder asphalt binder), base course, subbase course, and subgrade (*i.e.*, *in situ* soils) (Mallick and El-Korchi 2018a). The layered design of asphalt pavements allows the pavement structure to transfer loading from the surface course to the base and subbase layers by spreading out contact stress. Structurally, this reduces the stress intensity imposed on the lowest quality layer, the subgrade, to an acceptable level in order to prevent permanent deformation or damage (Federal Highway Administration and National Asphalt Pavement Association 2001; Islam and Tarefder 2020). The design of flexible pavements requires careful consideration of each of these layer properties in order to ensure adequate performance, reduce layer thicknesses, and improve cost-effectiveness (Little et al. 2018).

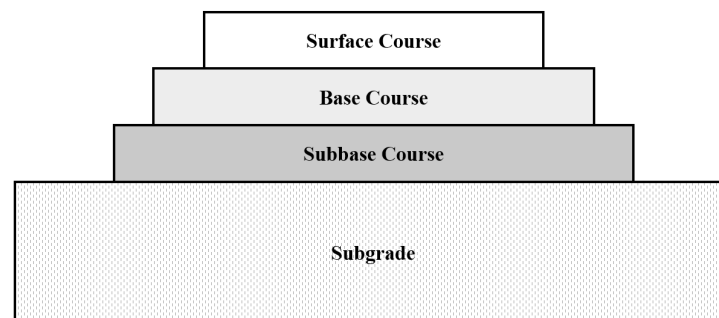


Figure 1.1: Typical cross-section of the layers of a pavement structure.

Typically, the surface course is constructed with the highest quality materials (*i.e.*, asphalt) and the material quality decreases with layer depth. The surface course responds elastically to

stresses and delivers a twofold structural benefit, providing sufficient stiffness against vehicle loading to prevent permanent deformation (*i.e.*, rutting or shoving), and providing sufficient flexural strength to resist cracking due to pavement pressures (*i.e.*, fatigue or thermal cracking) (Thom 2014). Moreover, the surface course provides protection for underlying layers and allows for drainage of surface and subsurface water (*Global Energy and CO₂ Status Report* 2018). The base course layer is typically constructed with aggregates and is designed to be stiff to resist deformation and permeable to route any water that enters the structure to properly drain. Depending on the condition of the subgrade, an additional subbase course of less costly soil or aggregate can be used to reduce stress intensity (Federal Highway Administration and National Asphalt Pavement Association 2001; Mallick and El-Korchi 2018a).

As a time-, temperature-, and age-dependent viscoelastic material, petroleum-based binders play a vital role in pavement infrastructure, forming the “glue” in the surface course (*i.e.*, asphalt) and in some cases, the base course (*i.e.*, as a stabilizer). Asphalt binders play a crucial structural role in pavements, which necessitates characterization of salient engineering properties in order to ensure adequate pavement placement and long-term durability (Speight 2016). Because the mechanical properties are known to vary considerably with the aforementioned variables, substantial research efforts have been aimed at developing test methods and standards that are specific to asphaltic binders.

Historically, empirical asphalt binder characterization techniques, based on observation and engineering experience, have been widely utilized in practice and in research because they are quick, inexpensive, and simple to perform (AAT 2011). The penetration test (ASTM D5), for example, is a measurement of binder consistency obtained by recording the penetration depth of a standard 100-g needle over a period of 5-s at 25°C (ASTM International. 2020b). A greater penetration value is associated with a less viscous (*i.e.*, softer) material whereas a lesser penetration value is associated with a more elastic (*i.e.*, stiffer) material. Binders are then graded according to penetration values specified in ASTM D946 that can be related to appropriate climate and traffic application (ASTM International 2020a).

Although empirical characterization methods are still routinely performed today on asphalt binders in practice and research, modern asphalt binders are more often graded using the Performance Grading (PG) asphalt binder specification (ASTM D6373), developed as part of research efforts by the 1987-1993 Strategic Highway Research Program (ASTM International

2021a). This system includes a specific experimental protocol for asphalt binders at multiple temperatures and ages which can be related to the expected pavement performance. Collaborative efforts between academia, industry, and government agencies to analyze the complex properties of asphalt binders has resulted in a highly developed research field.

Traditional Methods for Improving the Sustainability of Pavements

To reduce the environmental impacts and economic volatility of non-renewable petroleum-based asphalt binders, research has investigated the use of i) temperature reducing technologies and ii) recycled material use.

Temperature Reducing Technologies

Temperature reduction of asphalt mixtures is achieved through the use of foaming processes (*e.g.*, water, zeolites), organic additives (*e.g.*, waxes), or chemical additives (*e.g.*, polymers) (Rubio et al. 2012). By reducing working temperatures, fuel consumption and related emissions from incineration of fuel are reduced. With the use of temperature reducing technologies, asphalt mixtures can be classified into the following categories:

- **Hot Mix Asphalt (HMA)** - Manufactured at temperatures higher than 150 °C (300 °F)
 - **Warm Mix Asphalt (WMA)** - Manufactured between 100 - 140 °C (210 – 285 °F)
 - **Half Warm Mix Asphalt (HWMA)** - Manufactured between 60 - 100 °C (140 – 210 °F)
 - **Cold Mix Asphalt (CMA)** – Manufactured at temperatures lower than 40 °C (105 °F)
- (Cheraghian et al. 2020)

Significant energy reductions can be achieved with the use of these technologies, with WMA and HWMA using approximately 10-30% and 50% less energy, respectively (Rubio et al. 2013). Additionally, temperature reductions in pavement construction lead to lower asphalt plant emissions and fumes, reduced energy related costs, and improved workability and compaction efficiency. It is estimated that every 10 °C decrease in asphalt manufacturing temperature reduces fuel exhaustion by 1-L and CO₂ emissions by 1 kg/ton (Sukhija and Saboo 2021). Further, lower working temperatures improve the health and safety of workers by reducing asphalt fumes and the risk of burns or injury and allow for an extended paving window and quicker turnover to traffic after construction (Rubio et al. 2012).

Recycled Material Use

An additional approach to improve the sustainability of asphalt pavements is through the use of Reclaimed Asphalt Pavement (RAP) created from recycled end-of-life pavement structures. Aged, existing pavement structures (*i.e.*, aggregate and aged binder) are milled and crushed to create materials for new asphalt mixtures or subbase materials (Speight 2016). The use of RAP decreases the amount of virgin aggregate and petroleum-based binder needed for asphalt mixtures, thereby reducing raw material requirements and overall costs. Previous work has found that a mixture produced with 25% RAP reduced energy and material use by approximately 10% and 20-25%, respectively, as compared to a virgin asphalt mixture (Robinette and Epps 2010). RAP is one of the most recycled construction products and approximately 88% of available RAP in the USA was used for asphalt mixtures and unbound layers in 2018 (Tarsi et al. 2020).

The residual aged binder in RAP materials is often stiffer and more brittle as compared to virgin asphalt binders due to volatilization and oxidation of petroleum-based binders in service. As such, pavement mixtures including RAP materials often require a rejuvenating material in order to improve the stiffness and workability of mixtures. Rejuvenators can be categorized as paraffinic oils, aromatic extracts, tall oils, naphthenic oils, and triglycerides and fatty acids. Use of these rejuvenators requires additional testing to determine the quality and properties of the residual aged binder as well as the dosage and compatibility of the rejuvenating material with the recycled material (Kaseer et al. 2019). Over-dosing or utilizing an incompatible rejuvenator can lead to over-softening of mixes, and subsequent pavement durability problems like rutting and bleeding (Tarsi et al. 2020). Conversely, utilizing too high a quantity of RAP or under-dosing with rejuvenator can exacerbate fatigue and thermal cracking of an asphalt mixture due to the presence of stiff aged binders. Due to these pavement performance concerns, most agencies in the U.S. limit RAP content of an asphalt mixture to 15-20% (Kaseer et al. 2019).

Limitations and Opportunities

Although research oriented around temperature reduction technologies and recycled material use are incredibly promising, the aforementioned solutions represent an incremental improvement in regard to petroleum use and dependence rather than a paradigm shift away from petroleum and towards bio-renewable alternatives. Although these methods can reduce energy consumption and

minimize material needs, they are still rooted in petroleum use. With continued shifts away from petrochemical reliance, the construction industry has recognized this knowledge gap and has begun investigating novel materials to replace traditional petroleum-based binders.

Bio-Oils and Bio-Binders

Industrial shifts towards the use of renewable materials have led to an increased focus on the use of bio-oils as alternative asphalt binder materials. Bio-oils are produced through biochemical or thermochemical (*e.g.*, combustion, gasification, liquefaction, pyrolysis) processing of biomass (Ameri et al. 2023). Previous work has focused on the use of bio-oils created from renewable biomass sources including wood (He et al. 2019; Park et al. 2022; Raouf and Williams 2010a; b), animal waste (Fini et al. 2012), agricultural crops (Elkashef et al. 2017; Ghabchi and Pereira Castro 2021; Portugal et al. 2017), waste cooking oil (Ahmed and Hossain 2020; Zahoor et al. 2021), or vegetable-based oils (Chen et al. 2014; Elkashef et al. 2017; Portugal et al. 2017).

Using bio-oils to create bio-binders (*i.e.*, blending bio-oil and petroleum-binder) for asphalt can improve certain aspects of binder performance. Generally, bio-binders exhibit a lower softening point and improved viscosity, ductility, and low-temperature properties due to the high fluidity of bio-oils compared to petroleum-based binders (Park et al. 2022; Zhang et al. 2022). Due to these softening properties, bio-oils have been investigated as bio-renewable rejuvenators for asphalt mixtures with hardened, aged RAP (Ahmed and Hossain 2020; Chen et al. 2014; Zahoor et al. 2021; Zhang et al. 2020). A comprehensive review on the composition, preparation, and engineering characteristics of bio-binders can be found in Ameri et al (2023), Aziz et al. (2015), and Zhang et al. (2022).

Although promising, the body of literature on bio-oils has mainly focused on modifying traditional asphalt binders (<10% replacement). Fewer studies have explored the use of novel binder materials to act as extenders (25-75% replacement) or direct alternatives (100% replacement) for petroleum-based asphalt binders. Raouf & Williams studied the rheological properties of direct asphalt alternatives created from oakwood, cornstover, and switchgrass bio-oil (2010a; b). The bio-binders required heat treatment prior to use due to the presence of water and other volatile contents. The rheological behavior was similar in comparison to traditional petroleum-based asphalt binders, but the activation energy and viscosity susceptibility were higher, indicating more temperature susceptibility. Further, the low temperature performance grade was found to vary significantly. Peralta et al. (2013) investigated bio-based binders comprised of 85-

90% bio-oil from the pyrolysis of red oak residue and 10-15% rubber. This study reported similar findings regarding the comparable engineering properties of bio-binders but authors noted the need for additional research and field trials. Ample research is needed to fulfill knowledge gaps on alternative binder aging mechanisms, performance, polymer compatibility, and life cycle cost assessments before these materials can be widely implemented in modern construction (Zhang et al. 2022).

1.3 Biobased Materials from Biopolymers

Biopolymers

Biopolymers have recently gained traction as a sustainable substitute for synthetic or petroleum-based materials. Biopolymers are organic polymers that exist in nature or are produced naturally by living organisms (George et al. 2020). The general structure is that of a macromolecule composed of repeating monomer units including polynucleotides (*e.g.*, DNA, RNA), polypeptides, or polysaccharides (Singh et al. 2021). Biopolymers can be further classified by their structural backbone type as polyesters, polysaccharides, polycarbonates, polyamides, or vinyl polymers (George et al. 2020; Williams et al. n.d.).

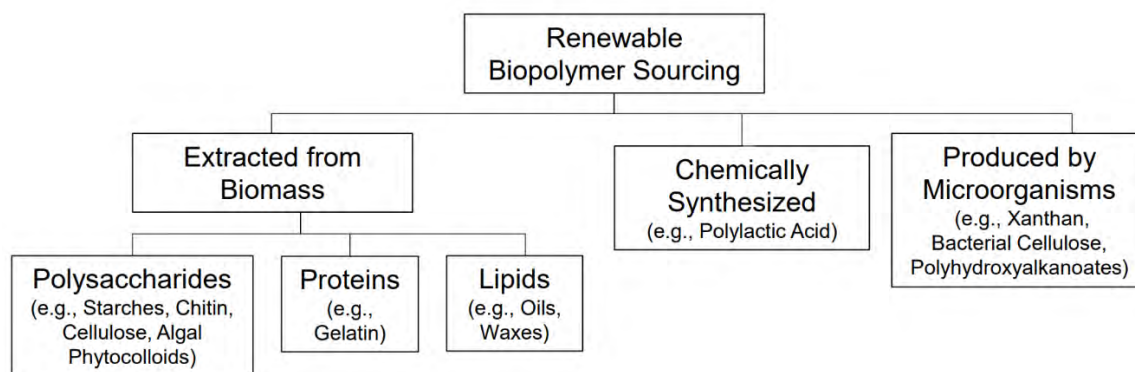


Figure 1.2: Renewable biopolymers categorized based on material source or method of production (Adapted from (Balart et al. 2021; Billington et al. 2014; George et al. 2020)).

More commonly, biopolymers are categorized based on the material source or the method of production used to derive them. Namely, common renewable biopolymers are 1) extracted from biomass, 2) produced from chemical synthesis using bioderived monomers, or 3) produced by microorganisms or through fermentation (Flórez et al. 2023; Nanda et al. 2022; Özçimen et al. 2017; Sukumaran and Gopi 2021). Biopolymers extracted from biomass sources include polysaccharides, proteins, or lipids (*i.e.*, polyamide structural backbone). Of these categories, the

most predominant and widespread is polysaccharides (Flórez et al. 2023; George et al. 2020). This general categorization and some of the most common biopolymers associated with each of these categories are shown in Figure 1.2.

Due to the generally low stiffness of biopolymers, reinforcement materials are often used to improve the mechanical properties (Engelsmann et al. 2012), forming valuable composites like fiber reinforced polymers, wood polymer composites (Agarwal and Gupta 2017), and engineered wood composites for both nonstructural and structural components (Islam et al. 2022; Nwankwo et al. 2023) (Plank 2004). (Hess and Srubar 2016; Srubar et al. 2012) Biopolymers have been investigated in tandem with biofibers and fillers like bamboo, cotton (Islam et al. 2022), hemp (Christian and Billington 2011), cellulose (Long Jiang et al. 2008; Semachai et al. 2018), saw dust (Mohan et al. 2021) (Srubar et al. 2012), and seaweed fibers (Barghini et al. 2010) to form insulation boards (Engelsmann et al. 2012), wall panels, roofing materials, flooring, or structural components (Vinod et al. 2020). Biopolymers act as a matrix to grip constituent materials together, which in turn aids in transferring load to the reinforcing materials rather than the biopolymer matrix alone (Islam et al. 2022; George et al. 2020).

Notably, biopolymers that are chemically synthesized or produced by microorganisms are more difficult to produce in large quantities as compared to biopolymers extracted from biomass due to the technology necessary to permit mass production and often have a higher cost of production (Yamane et al. 2014). Further, these technologies do not act as a side stream for biofuel or contribute to CO₂ adsorption during their production in the same manner that biopolymers derived from biomass do.

Synergistic Investments in Biomass Technologies

The large-scale cultivation and conversion of biomass presents benefits related to the promising generation of multiple products and the natural biological carbon capture illustrated during the growth period. Biomass can be converted to produce substances like ethanol, bio-oils, and biopolymers as valuable co-products of biofuel production, and remaining materials can be used as feed, fertilizer, or filler materials (Laurens et al. 2020). Anthropogenic CO₂ generation has led to an increased global interest in natural biological carbon capture and storage technologies using “sinks.” In the case of biomass, the photosynthetic growth of these materials converts water, sunlight energy, and most importantly, CO₂ from the environment, into oxygen (O₂) and organic matter- contributing to adsorption of harmful CO₂ from the atmosphere (Laurens et al. 2020).

The most widely studied biopolymers extracted from biomass are starches (*e.g.*, corn, grains, potato, sugarcane (Mori 2023)), cellulose, chitin, and algal phytocolloids. Cellulose is the most abundant biomass material in nature and forms the structural component of cell walls in plants including wood, cotton, flax, and jute (Nanda et al. 2022; Singh et al. 2021) (Flórez et al. 2023). Chitin represents the second most abundant biomass in the world (second to cellulose) (Kraan 2012) and is sourced from the exoskeleton or cell walls of arthropods including crustaceans, fish, insects, and microorganisms but is most commonly derived from discarded waste created from the seafood industry. (Kraan 2012; Sajid et al. 2018; Singh et al. 2021; Sukumaran and Gopi 2021). Lastly, the principle commercial algal phytocolloids include alginate, carrageenan, and agar (Gualtieri and Barsanti 2022). Alginate is extracted from the cell wall of brown seaweed whereas both carrageenan and agar are sourced from red seaweed (Özçimen et al. 2017) (Kraan 2012).

Biomass is commonly divided and classified into three generations. The first generation includes carbohydrate rich plants that are typically used as food sources and can include biomass from agricultural materials like corn, grains, and sugarcane. The second generation includes materials with a high lignocellulosic content that are typically not used as a food source like wood, cotton, flax, jute, or straw [52] (Flórez et al. 2023; Tanase et al. 2014). Lastly, the third generation includes materials from sources like algae or fungi (Oberti and Paciello 2022).

The adoption of first- and second-generation feedstocks raises concerns and criticisms. The cultivation of agricultural materials as a biomass feedstock can directly compete with valuable food supply chains and contribute to deforestation (Oberti and Paciello 2022). Further, the cultivation of terrestrial biomass feedstocks relies on the availability of arable land and the occupation of space that could be used for agriculture to meet increasing food demands (Flórez et al. 2023; Nanda et al. 2022; Sahoo et al. 2012; Singh et al. 2021)

The Potential of Algae

In contrast, algae, a third-generation biomass, has been identified as a promising candidate for the production of biomass as it does not require arable land or compete with agricultural food crops. Further, farming of algae does not require pesticides or fertilizers and allows for year-round production (Jalilian et al. 2020; Laurens et al. 2020; Sahoo et al. 2012) and the growth rate and yield of algae is typically much higher than that of terrestrial materials. For example, recent work has reported that macroalgae can grow *ten times faster* than farmed sugarcane (Sahoo et al. 2012).

Additionally, as compared to other biomass technologies, carbon capture through algae farming stands out due to the increased photosynthetic efficiency of algae as compared to terrestrial plants. Marine algae produce approximately half of the global atmospheric O₂ and accounts for as much as 50% of photosynthesis on earth (Paul et al. 2020; Sahoo et al. 2012; Sahoo and Seckbach 2015) and literature has estimated that the cultivation of 1 kg of dry algae biomass uses up to 1.83 kg of CO₂ (Paul et al. 2020).

Algal biomass is considered to be an excellent source for the production of products and polymers as it is biomass rich and does not contain difficult to process ligno-cellulosic components (Laurens et al. 2020). Although the composition of algae can vary for species and genera, the composition of algal biomass is generally carbohydrate rich and can be tailored by controlling growth conditions (Jalilian et al. 2020; Bernaerts et al. 2019; Sahoo et al. 2012). Biomass from algae can be processed to produce high value biopolymers, converted into biofuels or bio-oils (Jalilian et al. 2020), and residual wastes produced during processing can be further used as feed, fertilizer (Sahoo et al. 2012) or biofiller for composite materials (Freile-Pelegrín and Madera-Santana 2017).

Algae is a diverse classification of photosynthetic organisms encompassing over 72,000 strains of unicellular microalgae and multicellular macroalgae (i.e., seaweed). Macroalgae are generally classified into three groups based on photosynthetic pigment- Namely, brown (phaeophyta), green (chlorophyta), or red (rhodophyta) algae. Microalgae are categorized similarly, but also include diatoms, prokaryotes, or dinoflagellates (Jalilian et al. 2020). Commercially, approximately 220 species are utilized for food or phytocolloid (i.e., alginate, agar, or carrageenan) production (Sahoo and Seckbach 2015).

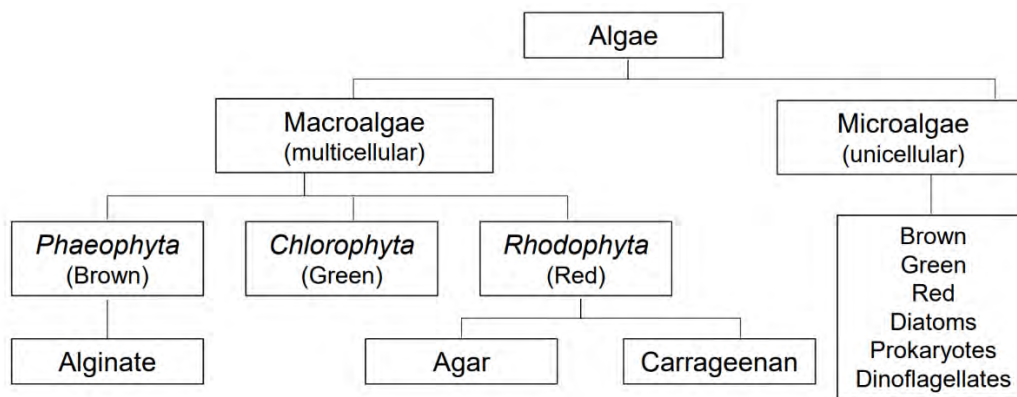


Figure 1.3: General classification of algae and algal phytocolloids produced from macroalgae.

Limited literature has evaluated algae derived bio-binders. Audo et al. (2015) used hydrothermal liquefaction to produce a water-insoluble viscous material from *Scenedesmus sp.* microalgae residues, which showed some promise (*i.e.*, rheologic and viscoelastic similarities) as an alternative road binder. Pahlavan et al. (2020) examined a bio-oil produced from the co-liquefaction of protein rich algae from the *Galdieria sulphuraria sp.* and lipid rich swine manure. This “swilgae” bio-oil was found to be more effective at bio-rejuvenation than algae or swine manure bio-oils alone making it a good candidate for use in RAP mixtures. Further work (Pahlavan et al. 2021) found that swilgae bio-oil can restore aged bitumen morphology and increase resistance to moisture damage due to a more balanced molecular content of nitrogen rich compounds and less hydrocarbon chains. Li et al. (2019) investigated bio-binder with 20% bio-oil prepared by esterification and vacuum distillation of seaweed in addition to styrene-butadiene-styrene (SBS). Seaweed bio-binder was found to improve the performance related to high temperature rutting and low temperature resistance to fracture.

1.4 Agar

One algae-based biopolymer that might be well-suited for construction applications is agar. Agar is a linear polysaccharide extracted from red seaweed belonging to the *Rhodophyceae* class (Nishinari and Fang 2017). The Rhodophyceae class of algae comprises approximately 6,500 to 10,000 species of macroalgae that grow in the upper sunlit aquatic euphotic zone of cold or warm marine environments (Gualtieri and Barsanti 2022; Sahoo and Seckbach 2015). The major commercial agarophytes, or agar-containing seaweed species, belong to the genera *Gracilaria*, *Gelidium*, *Gracilariopsis*, and *Pterocladia* (Gualtieri and Barsanti 2022; Freile-Pelegrín and Madera-Santana 2017; Sahoo and Seckbach 2015; Zia et al. 2017).

Agar fulfils a function analogous to cellulose in terrestrial plants (Madera-Santana et al. 2010) and confers stiffness to cell walls of marine algae, providing protection against predators and environmental stressors (*e.g.*, salinity, pH, and temperature), as well as providing the flexibility necessary to resist water currents and wave motion (Lee et al. 2017). Agar has passed all toxicological, teratological, and mutagenic testing through the United States Food and Drug Administration and is biologically inert, biodegradable, and non-toxic (Armisen et al. 2009; Mostafavi and Zaeim 2020).

Agar is the strongest gelling hydrocolloid (Freile-Pelegrín and Madera-Santana 2017) composed of agarose and agaropectin, with agarose acting as the component responsible for

gelling properties and agarpectin acting as a structural support (Zia et al. 2017). When dissolved in water, agar gels via hydrogen bonding between agarose molecules, which create a double-helix structure [79]. This process forms a transparent, thermoreversible gel that exhibits hysteresis, gelling at temperatures between 38 and 45 °C and melting at temperatures from 85 to 90 °C [80]. Due to its very high hysteresis (*i.e.*, 45 °C) and gel strength at minimal concentrations, low concentrations of agar (*i.e.*, 0.5-2.0%) are commonly used as additives to food, microbiology culture media, or other pharmaceutical applications (Lee et al. 2017).

Agar extraction involves a process of seaweed pretreatment followed by extraction, filtration, and dehydration (Freile-Pelegrín and Madera-Santana 2017; Gualtieri and Barsanti 2022; Zia et al. 2017). During pretreatment, red seaweed is harvested and cleaned to remove any surface impurities and often dried and milled to increase surface area for processing. Additional pretreatment processes can vary depending on the species of seaweed utilized. For example, *Gracilaria* is more sulfated than other species and often requires alkali treatment to improve the proportion of 3,6 anhydrogalactose in agarose components and the resulting gel strength of extracted agar (Freile-Pelegrín and Madera-Santana 2017; Gualtieri and Barsanti 2022). Following pretreatment, agar is extracted by dissolving treated seaweed in boiling water, often under pressure. This solution is then filtered to remove residual seaweed or impurities and the filtrate is cooled and allowed to form a gel. Gelled filtrate is then dried to remove excess moisture and the resulting solid is often re-milled to produce powdered or flaked agar (Özçimen et al. 2017).

Agarophytes grow naturally in coastal regions attached to substrates like bedrock and boulders but can be cultivated in marine farms using an off bottom monoline, raft, or single longline rope farming system. Agarophytes are an attractive candidate for cultivation in offshore marine farming due to their high consumption of CO₂ during growth. For example, *Gracilaria chilensis* biomass productivity has been reported as 57 dry tons acre⁻¹ year⁻¹ as compared to 40 fresh tons acre⁻¹ year⁻¹ for sugar cane, one of the highest yielding terrestrial crops. At the reported biomass productivity rate of 57 dry tons acre⁻¹ year⁻¹, it is estimated that the cultivation of *G. chilensis* would have a CO₂ capture potential of 62 tons acre⁻¹ year⁻¹ (Laurens et al. 2020). Additionally, the cultivation of seaweed like agarophytes can reduce eutrophication and purify marine farming waters by consuming harmful nitrates and phosphates (Taelman et al. 2015).

Although seaweed is an attractive candidate for cultivation in offshore marine farming, it is generally underutilized in the United States. In terms of dry mass, the global marine agronomy

produces 30 million metric tons per year and the U.S. only produces approximately 1.4% of that material (Laurens et al. 2020). China and Indonesia are currently the world leaders in agar production comprising 54% and 28% of the production market, respectively, followed by the Philippines (7%) and Korea (4%). The global market for agar is anticipated to grow approximately 5% from 2016 to 2025 (Chudasama et al. 2021) which has led to increased cultivation in European countries (Kraan 2012; Taelman et al. 2015) in addition to coastal regions of the United States like California (Gualtieri and Barsanti 2022). Work by Laurens et al. (2020) notes the untapped potential of the exclusive economic zone (EEZ) or the ocean area in which the US has jurisdiction over natural resources. Literature estimates that the use of 2.5% of the EEZ for marine agriculture would result in carbon capture capable of displacing between 10 and 20% of U.S. fossil energy emissions.

1.5 Points of Departure

Out of highlighted categories of petroleum-based construction materials, asphalt binders used in pavement construction are one of the most prevalent. Although a good amount of literature exists on biobased material alternatives for petroleum-based asphalt binders, the majority of this work has focused solely on bio-oils and the use of small replacement quantities rather than direct replacements. Further, much of the literature reviewed herein has focused on the creation of alternatives from first and second-generation biomass sources (*i.e.*, terrestrial sources) rather than promising third generation biomass sources like algae. Algal biomass represents an incredible opportunity for the large-scale adsorption of CO₂ and the production of multiple high value products without creating competition with agriculture or land use.

Biopolymers have illustrated promise as alternative plastics and soil stabilization materials but are largely underutilized in civil engineering applications. Some of the major concerns in regard to developing biobased building or construction materials are related to undesirable hydrophilic behavior, biological deterioration due to microorganisms, and thermal stability. These risks pose challenges related to the appearance and mechanical properties of biobased materials as well as impact human health and the service life of built infrastructure these materials are integrated into (Stefanowski et al. 2017).

Moreover, researchers note a lack of technoeconomic and long-term performance data to support the widespread use of biopolymers in civil engineering applications. Substantial research efforts have been aimed at developing test methods and standardized testing protocol that is

specific to pavement performance. Despite how well developed this research field is, very little literature has evaluated biopolymers within the explicit context of transportation infrastructure, and more specifically, in the context of petroleum-based asphalt binders. Connecting novel biopolymer materials like agar with engineering design and standardization will require research that leverages the established knowledge base related to pavement binders and communicates findings in a contextualized, meaningful way.

1.6 Report Overview

The intention of this project is to investigate the interesting nexus between petroleum-based pavement materials, algae-based technologies, and biopolymers. In particular, the primary objective of this work is to characterize highly concentrated agar gels and evaluate their suitability as direct replacement materials (i.e., 100% replacement) for traditional, petroleum-based construction materials. When applicable and able, empirical asphalt binder characterization techniques were implemented in addition to traditional materials testing techniques to communicate and contextualize research results. This work investigates the most commonly noted durability concerns and mechanisms associated with biopolymers and evaluates methods to improve these functional properties. Agar-based materials are combined with standardized pavement aggregate in order to directly compare composite mechanical and microstructural properties.

To specifically address the research objective and points of departure noted, the following studies and research questions are posed and structured in three chapters:

Chapter 2 summarizes work evaluating the durability and engineering properties of agar for pavement applications. The research questions addressed in this chapter include: Can high concentration agar gels act as direct replacements for petroleum-based products used in the construction industry? How do the durability and engineering characteristics of agar-based materials compare to traditional petroleum-based construction materials and asphalt binder used in pavement construction?

Chapter 3 characterizes and models moisture diffusion of agar-based materials. The research questions addressed in this chapter include: Can the hydrophobicity of agar-based materials be improved through chemical or physical modification methods? What is the relative efficacy of

chemical or physical modification methods? Can the diffusion behavior of agar-based materials be modeled in order to produce predictions of moisture behavior?

Chapter 4 characterizes the mechanical and microstructural properties of agar-based composites for pavement applications. The research questions addressed in this chapter include: How do the microstructure and mechanical properties of agar-based composites compare to traditional engineering materials and biobased composites? What engineering applications can agar-based materials constitute a sustainable material alternative?

Chapter 5 summarizes the conclusions of this research and the suggested future work related to agar-based materials. The experimental methods utilized to address the research questions posed are summarized in Table 1.1.

Table 1.1: Organization of project report and summary of methods used

Chapter		Characterization Methods
2	Agar-Based Materials: Evaluating Durability and Engineering Properties of Agar for Pavement Applications	Thermogravimetric Analysis (TGA) Moisture Sorption Characteristics (ASTM D570) Biodeterioration Resistance (ASTM G21) Rotational Viscosity (ASTM D4402) Dynamic Shear Rheometry (ASTM D6648) Penetration (ASTM D5) Bending Beam Rheometry (ASTM D6648)
3	Chemical and Physical Modification of Agar: Classification and Modeling of Anomalous Diffusion Behavior	Fourier Transform Infrared (FTIR) Spectroscopy Moisture Sorption Characteristics (ASTM D570) Diffusion Classification and Modeling
4	Mechanical and Microstructural Characterization of Agar-Based Composites	X-Ray Tomography Testing Compressive Strength Testing Resilient and Elastic Moduli Characterization

2. Agar-Based Materials – Evaluating Durability and Engineering Properties for Pavement Applications

2.1 Introduction

Petroleum is an integral component in the production of numerous industrial and domestic commodities. Within the construction industry, products derived from petroleum include plastics (*e.g.*, polyvinyl chloride, high density polyethylene) (Billington et al. 2014), adhesives (*e.g.*, polyurethane, epoxies, poly(vinyl acetate), silicones) (Magalhães et al. 2019), and binders (*e.g.*, asphalt for roadways, roofing, coatings, and waterproofing) (Speight 2016). Out of the aforementioned categories of petroleum-based construction materials, asphalt binders used in pavement construction are one of the most prevalent. Roads and highways represent the largest sector of national assets (Aziz et al. 2015) and approximately 95% of these pavements are petroleum-based (Asphalt Paving Association of Iowa 2020). Despite the widespread use and acceptance, petroleum-based materials are associated with negative environmental impacts due to petroleum sourcing, refining, and manufacturing (Eneh 2011). Further, petroleum is a nonrenewable resource, and oil reserves are dwindling, resulting in significant price fluctuations (Cui et al. 2020; *Reclaimed Asphalt Pavement in Asphalt Mixtures: State of the Practice* 2011; Thives and Ghisi 2017).

To meet the global environmental need for sustainable and resilient construction materials, the construction industry must begin to shift to direct alternatives (*i.e.*, 100% replacement) for petroleum-based materials. Biopolymers have been noted as potential sustainable substitutes for synthetic or petroleum-based construction materials due to their biorenewability and promising carbon capture potential (Laurens et al. 2020). Previous work has investigated the use of biopolymers as bioadhesives for engineered wood products (Hess and Srubar 2016; Shalbafan and Thoemen 2020) fiberboards, insulation materials (Rabbat et al. 2022), plasters, and natural fiber composites (Christian and Billington 2011; Losini et al. 2021) as well as soil stabilizing materials for clayey and silty soils (Bozyigit et al. 2023; Chang et al. 2015a; Huang et al. 2021b; Khatami and O’Kelly 2013; Smitha and Sachan 2016).

One algae-derived biopolymer that may be well suited as an alternative material for civil engineering applications is agar. Agar is a linear polysaccharide extracted from red seaweed

belonging to the Rhodophyceae class where it fulfills a function analogous to cellulose in terrestrial plants (Madera-Santana et al. 2010). Agar gels form *via* hydrogen bonding between agarose molecules (Mostafavi and Zaeim 2020), resulting in a transparent, thermoreversible material that exhibits hysteresis, gelling at temperatures between 38 and 45 °C and melting between 85 to 90 °C (Armisen et al. 2009). Agar has passed all toxicological, teratological, and mutagenic testing through the United States Food and Drug Administration and is biologically inert, biodegradable, and non-toxic (Armisen et al. 2009; Mostafavi and Zaeim 2020).

Although biopolymers like agar are promising material alternatives for petroleum-based materials, they are largely underutilized in civil engineering applications due to uncertainty regarding long term performance and durability (Huang et al. 2021b). Additionally, there is a lack of systematic methodology available for predicting performance, reporting material characteristics, and ultimately incorporating biopolymer materials incorporation into engineering design (Losini et al. 2021; Vinod et al. 2020). Literature highlights the need for contextualized data that can be used to draw direct comparisons between novel biopolymer-based materials and traditional, well studied and characterized engineering materials.

2.2 Research Objectives

The objective of this work is to study the suitability of high concentration agar gels as a direct replacement material for traditional petroleum-based materials used in the construction industry. To achieve this goal, this research follows a two-step approach. First, this study evaluates the durability of agar-based materials for comparison to traditional petroleum-based construction materials. Second, the study explores the potential of agar-based binders to be used in a particular construction application, namely as an alternative binder in pavement materials.

Durability Evaluation

In this study, durability is evaluated in terms of thermal stability (*i.e.*, thermogravimetric analysis), fungal biodeterioration, and moisture sorption characteristics. These durability metrics were chosen based on limitations of biopolymers and biobased materials noted in literature. Thermal, moisture, and biodeterioration durability are some of the leading durability concerns that impose challenges in accepting and implementing biobased materials in widespread industrial applications (Billington et al. 2014). Relatedly, the undesirable hydrophilic behavior of biopolymers can provide pathways of moisture for the introduction of harmful microorganisms leading to

biodeterioration (Billington et al. 2014; Rabbat et al. 2022). The aforementioned mechanisms can negatively impact the appearance and mechanical properties of biobased materials as well as impact human health and the service life of built infrastructure these materials are integrated into (Stefanowski et al. 2017).

Evaluation of Engineering Characteristics for Pavement Applications

Standard asphalt binder testing methods were implemented to compare the engineering properties of agar with those of asphalt. This methodological approach was chosen to allow for direct comparison to a reference petroleum-based material used in civil engineering applications. Rotational viscosity (RV), dynamic shear rheometry (DSR), bending beam rheometry (BBR), and penetration testing were conducted on agar-based materials. RV, DSR, and BBR results were subsequently compared to performance grade (PG) specifications (ASTM International 2021a) and penetration results were compared to penetration grade specifications (ASTM International 2020a). Experimental protocols used in the study are summarized in Table 2.1.

Table 2.1: Experimental characterization methods for agar-based materials.

Characterization	Test	Test Measure	Test comparison
Durability Evaluation	Thermogravimetric Analysis	High temperature durability	Results compared to literature
	Fungal Resistance (ASTM G21)	Biodeterioration	
	Moisture Sorption (ASTM D570)	Moisture sorption characteristics	
Evaluation of Engineering Characteristics for Pavement Applications	Rotational Viscosity (ASTM D4402)	Workability	Results compared to Grade (PG) specification (ASTM D6373)
	Dynamic Shear Rheometry (ASTM D7175)	Rutting resistance	
	Bending Beam Rheometry (ASTM D6648)	Low temperature stiffness	
	Penetration (ASTM D5)	Consistency, hardness	Results compared to penetration specification (ASTM D946)

2.3 Materials and Methods

Materials

Agar powder, reagent-grade limestone (calcium carbonate, CaCO_3) with a particle size $< 250 \mu\text{m}$, lab-grade glycerol, and Ottawa sand were supplied by Sigma Aldrich (St. Louis, MO, USA), Research Products International (Mt Prospect, IL, USA), Fisher Scientific (Waltham, MA, USA), and Gilson (Middleton, WI, USA) respectively. Fungal cultures were supplied by the American Type Culture Collection (ATCC, Manassas, VA, USA).

Methods

Sample Preparation

Samples were prepared at three agar concentrations (*i.e.*, 5, 7.5, and 10% w/w relative to deionized water) without and with glycerol and without and with limestone additives. As these are relatively high agar concentrations and agar materials have a high degree of crystallinity and rigidity (Smitha and Sachan 2016), glycerol was investigated due to reported plasticizing effects on agar (Arham et al. 2018). In order to increase the dimensional stability of agar-based materials, ground limestone was investigated as a filler material. Limestone was chosen as a potential filler material because it is widely available and already utilized in the construction industry (*i.e.*, crushed for use as a subbase material, ground for use as a raw material in cement production).

A subset of samples contained glycerol (40% w/w of agar), limestone (15% w/w), or both glycerol and limestone, resulting in a total of twelve samples. Mixture formulations are summarized in Table 2.2. The sample naming convention is the letter A (for agar) followed by the %w/w agar-to-water concentration. Sample additives are denoted by a C (for control formulations without glycerol or limestone), G (for glycerol), and L (for limestone). For example, sample A7.5-GL indicates a 7.5% agar-to-water concentration with glycerol and ground limestone.

Table 2.2: Sample nomenclature and mixture formulations.

Sample	Deionized Water (ml)	Agar (g)	Glycerol (ml)	Limestone (g)
A5-C	200	10	-	-
A5-G	200	10	3.2	-
A5-L	200	10	-	30
A5-GL	200	10	3.2	30
A7.5-C	200	15	-	-
A7.5-G	200	15	4.8	-
A7.5-L	200	15	-	30
A7.5-GL	200	15	4.8	30
A10-C	200	20	-	-
A10-G	200	20	6.4	-
A10-L	200	20	-	30
A10-GL	200	20	6.4	30

To prepare samples, 200 ml of deionized water was added to 500 ml media bottles. The appropriate amount of agar powder (*i.e.*, 10, 15, or 20-g) was dissolved at ambient laboratory conditions (22 ± 2 °C) under continuous agitation using a magnetic stir bar. The appropriate amount of glycerol or limestone was then added and the sample was agitated until all constituent materials were well dispersed. The media bottles were then placed in an SK101C Yamato autoclave (Santa Clara, CA, USA) and heated to 100 °C for 20 min. After autoclaving, individual media bottles were placed in a heated water bath at 90 ± 5 °C until sample molding or testing.

Durability Analysis

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was performed with a TA Discovery 5500 (TA Instruments, New Castle, DE, USA). Agar samples were poured into 55 mm diameter molds to create disc-shaped samples with a depth of approximately 10 mm. After gelling for 30 min at ambient laboratory conditions (22 ± 2 °C), samples were placed in incubation at 30 °C until samples reached constant mass (*i.e.*, < 1% change in mass in 1 hour). A 10-20 mg sample was collected from the center of the dehydrated agar sample and placed in a platinum pan. Testing was conducted using continuous nitrogen gas flow at a rate of 10 ml/min. Samples were acclimated at 30 °C and then heated to 950 °C using a heating rate of 10 °C/min. The temperature and sample weight loss were recorded for analysis. Each sample was tested in duplicate.

Fungal Biodeterioration

Resistance to fungal biodeterioration was determined according to ASTM G21 (ASTM International 2021b). The following fungal strains, shown in Figure 2.1, were used to create the testing spore solution: *Aspergillus brasiliensis* (ATCC 9642), *Penicillium funiculosum* (ATCC 11797), *Chaetomium globosum* (ATCC 6205), *Trichoderma virens* (ATCC 9645), and *Aureobasidium pullulans* (ATCC 15233).

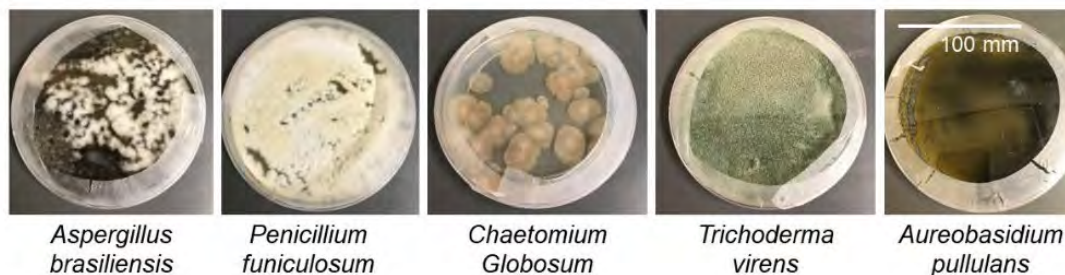


Figure 2.1: Fungal strains cultivated to create ASTM G21 spore solution.

Nutrient-salts agar solutions were prepared according to ASTM G21. Solutions were poured into sterile petri dishes measuring 150 mm in diameter and 15 mm in depth and allowed to gel. Agar samples to be tested for fungal resistance were prepared according to Table 2.2 and poured into 55 mm diameter sample molds to create disc shaped samples with a depth of approximately 10 mm. Samples were allowed to gel for approximately 30-min at ambient conditions (22 ± 2 °C) and then placed on the top surface of the nutrient-salts agar plates. Each nutrient-salts agar and agar test sample were inoculated by spreading a spore solution over the entire exposed surface with a sterile cell spreader. A negative control (*i.e.*, blank nutrient-salts agar plates without a sample, denoted B-) and a positive control (*i.e.*, nutrient-salts agar plates with sterilized cellulose paper, denoted C+) were also prepared to validate the experiment. Samples were sealed with parafilm and incubated at 30 °C for 28 days. Photos and observations were recorded every 7 days.

Biodegradation is a biochemical process where microorganisms metabolically degrade complex materials into natural compounds such as water, CO₂, and biomass (Folino et al. 2020). The ASTM G21 (ASTM International 2021b) procedure is designed to provide an environment where heterotrophic microorganisms are provided with all the necessary components for growth (*e.g.*, moisture, salts, minerals) except for excess organic carbon. At the end of the 28-day testing period, samples were visually examined and evaluated by using a rating of 0-4 based on the

percentage of sample area covered in fungal growth. This rating system is shown in Table 2.3. All samples were tested in triplicate.

Table 2.3: Rating system used for the evaluation of agar-based material fungal resistance.

<i>Observed Growth on Specimens</i>	<i>Rating</i>
None	0
Traces of growth (< than 10% of sample area)	1
Light growth (10-30% of sample area)	2
Medium growth (30-60% of sample area)	3
Heavy growth (60% to complete coverage)	4

Moisture Sorption

The moisture sorption properties of agar-based materials were determined according to a modified ASTM D570 procedure (ASTM International 2022). The procedure was modified minimally by lowering the conditioning temperature from 50 °C to 30 °C. Fresh agar samples were poured into 55-mm diameter molds to create disc-shaped samples with a depth of approximately 10-mm. After gelling for 30-min at ambient laboratory conditions, samples were weighed to determine the fresh sample mass and then conditioned in incubation at 30 °C until reaching constant mass, defined as < 1% decrease in mass in a 24-hr period.

Moisture sorption testing was conducted in 20 ± 2 °C distilled water. At various intervals, samples were removed from the distilled water, the surface moisture was removed, and the mass of each sample was recorded. Sample masses were recorded every 24-h until the sample reached constant mass. The moisture content was calculated using Equation 2.1:

$$\text{Equilibrium Moisture Content (\%)} = \frac{m - m_c}{m_c} * 100\% \quad \text{Equation 2.1}$$

where m is the final immersed sample mass upon equilibration and m_c is the conditioned sample mass. After reaching constant mass in immersion testing, samples were reconditioned in incubation at 30 °C until again reaching constant mass, cooled for approximately 1-h, and reweighed. The percentage of matter lost, M_{Loss} , during immersion was calculated for each sample using Equation 2.2:

$$M_{Loss} (\%) = \frac{m_c - m_{rc}}{m_c} * 100\% \quad \text{Equation 2.2}$$

where m_{rc} is the sample mass after reconditioning. Each agar-based binder was tested in triplicate.

Characterization of Engineering Properties for Pavement Applications

Rotational Viscosity

The rotational viscosity (RV) of fresh-state agar-based materials was measured according to a modified ASTM D4402 procedure (ASTM International 2015a). The procedure was modified by using a rotational rheometer instead of a Brookfield viscometer and lowering the testing temperature from 135 °C to 80 °C. The performance grade (PG) specification (ASTM International 2021a) utilizes an RV testing temperature of 135 °C to mimic the conditions of asphalt during construction. However, prolonged incubation of fresh-state agar at temperatures above 80 °C has been shown to impact structural and mechanical properties of agar gels (Mao et al. 2017). Thus, RV was measured at 80 °C in order to represent agar temperature compatibility and preparation temperatures more accurately.

Fresh-state samples were immediately loaded into an MCR 301 rotational rheometer (Anton Paar, Graz, Austria) with 25-mm diameter stainless steel parallel plate geometry and a sample gap of 1.0-mm. The top plate was cross-hatched to minimize slip. After equilibration at 80 °C for 15-min, a constant shear rate of 20 s⁻¹ was applied for 3 min. Three consecutive RV measurements were recorded at 1-min intervals for each sample. These data were averaged to yield final reportable RV values. Each sample was tested in triplicate.

The RV of asphalt binders is typically characterized to predict the workability at specific handling, mixing, and application temperatures to ensure that the mixtures can be properly mixed and compacted to the required pavement density and smoothness (ASTM International 2015a). To this end, asphalt mixtures with inadequate workability are often difficult to compact properly, resulting in a lower pavement strength, higher air void content, reduced moisture resistance (Jagan Gudimetla et al. 2004), and loss of service life (Dessouky et al. 2013; Linden et al. 1989). Results were compared to PG specifications that require a $RV < 3 \text{ Pa}\cdot\text{s}$ (ASTM International 2021a).

Dynamic Shear Rheometry

A dynamic shear rheometer (DSR) was used to determine the high-temperature rheological properties and rutting behavior of agar-based materials. The complex modulus (G^*), phase angle (δ), and rutting factor, defined were determined using an MCR 301 rotational rheometer (Anton Paar, Graz, Austria) with 25-mm diameter stainless steel parallel plates. The rutting factor was calculated at various testing temperatures using Equation 2.3:

$$\text{Rutting Factor} = \frac{G^*}{\sin(\delta)} \quad \text{Equation 2.3}$$

Cross-hatched top and bottom plates were used to minimize slip. The testing temperature ranged from 10-80 °C using a heating rate of 2 °C/min.

Freshly prepared agar samples were poured into 55-mm diameter molds to create disc-shaped samples with a depth of approximately 5-mm. After gelling for 30-min at ambient laboratory conditions (22 ± 2 °C), samples measuring 25-mm in diameter and 1-mm in thickness were taken from the center for testing. Beginning at the lowest testing temperature (i.e., 10 °C), samples were acclimated at the testing temperature for 20-min and then subjected to oscillatory shear flow at 12% strain amplitude and a rotational frequency of 10 rad/s. These parameters were chosen in order to reflect standard unaged asphalt binder rheological testing protocol (i.e., ASTM D7175) (ASTM International 2015b). A constant axial force of 0.5 N was applied to account for thermal expansion and contraction. Each sample was tested in duplicate.

To resist rutting, an asphalt binder should be both stiff and elastic to maintain shape and recover in response to repeated traffic loading (Mallick and El-Korchi 2018b; Thom 2014). To this end, a higher complex modulus (G^*) and a lower phase angle (δ) are advantageous, resulting in the maximization of the rutting factor. To ensure adequate stiffness to resist permanent deformation (i.e., rutting), performance grade specifications (ASTM International 2021a) require the rutting factor to exceed 1.00 kPa at the upper pavement design temperature for unaged binders. Agar-based materials were compared to this specification.

Penetration

Penetration was determined according to ASTM D5 (ASTM International 2020b) using a Universal Penetrometer (Humboldt, Elgin, IL, USA). Fresh samples were poured into seamless tin cups measuring 80-mm in diameter and 50-mm in depth, covered, and stored for less than 24-h in refrigeration at 4 ± 2 °C until testing in order to prevent desiccation. Samples were then submerged in a 25 °C water bath for 30-mins prior to testing, and they remained immersed in 25 °C deionized water for the duration of testing to ensure temperature stability. This experimental setup is illustrated in Figure 2.2. Results were compared to penetration grading standards (ASTM D946), which specify acceptable penetration grades in bins that range from 40 to 300 for unaged asphalt binders (ASTM International 2020a). Triplicate penetration readings were collected for each sample.

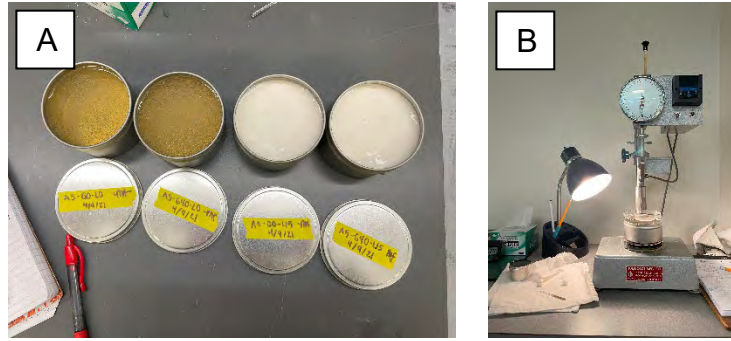


Figure 2.2: (A) Agar-based materials prepared in standardized tins for penetration testing. (B) Penetration setup with sample submerged in water bath.

Penetration is a measurement of binder consistency, and lower penetration values typically correspond to stiffer material behavior while higher penetration values typically correspond to softer material behavior (ASTM International 2020a). Traditional asphalt binders with high penetration values (i.e., softer consistency) are typically used in cold climates to combat pavement cracking. Conversely, binders with low penetration (i.e., stiffer consistency) are typically used in warmer climates where a binder is expected to resist permanent deformation at high temperatures (Williams 1961).

Bending Beam Rheometry

Bending beam rheometry (BBR) testing is conducted on aged asphalt binder and used as a fundamental low service-temperature criterion in performance grade (PG) specification (ASTM International 2021a). This test applies a constant load to the mid-point of a simply supported beam of asphalt binder and measures the strain rate of the binder sample as it deflects. From these results, the stiffness at low pavement design temperatures can be determined (Southern 2015). Performance grade (PG) specification defines thresholds for the creep stiffness (S) and the slope of the stiffness curve (m -value) at 60-s (ASTM International 2021a). These thresholds are set to ensure that a binder can dissipate pavement stresses and recover from repeated loading. BBR testing is typically conducted on aged petroleum-based binders as they are stiffer than virgin binders and more prone to low-temperature thermal cracking (Speight 2016). Similar stiffening effects are expected with aging of agar-based binders due to dehydration. To mimic the aged conditions of petroleum-based binders in BBR testing, agar-based binders were aged through acclimation in ambient laboratory conditions until constant mass was achieved.

The low temperature flexural creep stiffness of agar-based materials was determined with a Cannon TE-BBR SD Bending Beam Rheometer (BBR) by ASTM D6648 (ASTM International

2016). 10% w/w agar samples and samples without limestone filler were not tested due to inadequate workability and dimensional instability, respectively. Four sample types were tested in BBR- Namely, A5-L, A5-GL, A7.5-L, and A7.5-GL. Fresh samples were mixed by hand with Ottawa sand at a ratio of 2:1 (*i.e.*, 100 ml of binder per 50 g of sand) for approximately 1-min until materials were well dispersed. Ottawa sand was added to aid in dimensional stability and ensure test specimens were correctly sized for the BBR apparatus.

The sand and agar mixture were poured into standard aluminum molds to create samples measuring 6.25 x 12.5 x 127 mm (0.25 x 0.50 x 5.00 in). After gelling for 30-mins, samples were removed from the aluminum molds and remained in ambient laboratory conditions (22 ± 2 °C) until constant mass (*i.e.*, < 1% change in mass in 1 hour) was achieved. BBR testing was completed at -12 °C in a temperature-controlled ethanol bath. Samples were acclimated in the ethanol bath at the testing temperature for 30-mins prior to testing and tested in triplicate. Results from testing were compared to the PG specification (ASTM International 2021a) which requires a creep stiffness (S) less than 300 MPa and the slope of the stiffness curve (m) to be greater than 0.300 at the 60-s testing mark.

2.4 Durability Analysis Results

Thermogravimetric Analysis

All agar-based material formulations were thermally stable up to 210°C, followed by a steep mass loss due to decomposition of agar and glycerol (Figure 2.3). Similar thermal behavior for agar composite materials has been reported previously (Madera-Santana et al. 2010; Nishinari and Fang 2017). For the agar-based formulations containing limestone, a second steep mass loss was observed beginning at 650°C due to the decarbonation of limestone. Although the thermal stability of agar-based materials appeared to be minimally affected by agar concentration, the addition of both glycerol and limestone substantially impacted thermal stability. Specifically, glycerol addition resulted in a decrease in thermal stability and limestone addition resulted in an increase in thermal stability. The 5%, 7.5%, and 10% agar-based binder control formulations retained 29.60%, 29.54%, and 30.59% of their original mass, while limestone formulations retained 43.55%, 44.33%, and 39.45% of their original mass, respectively.

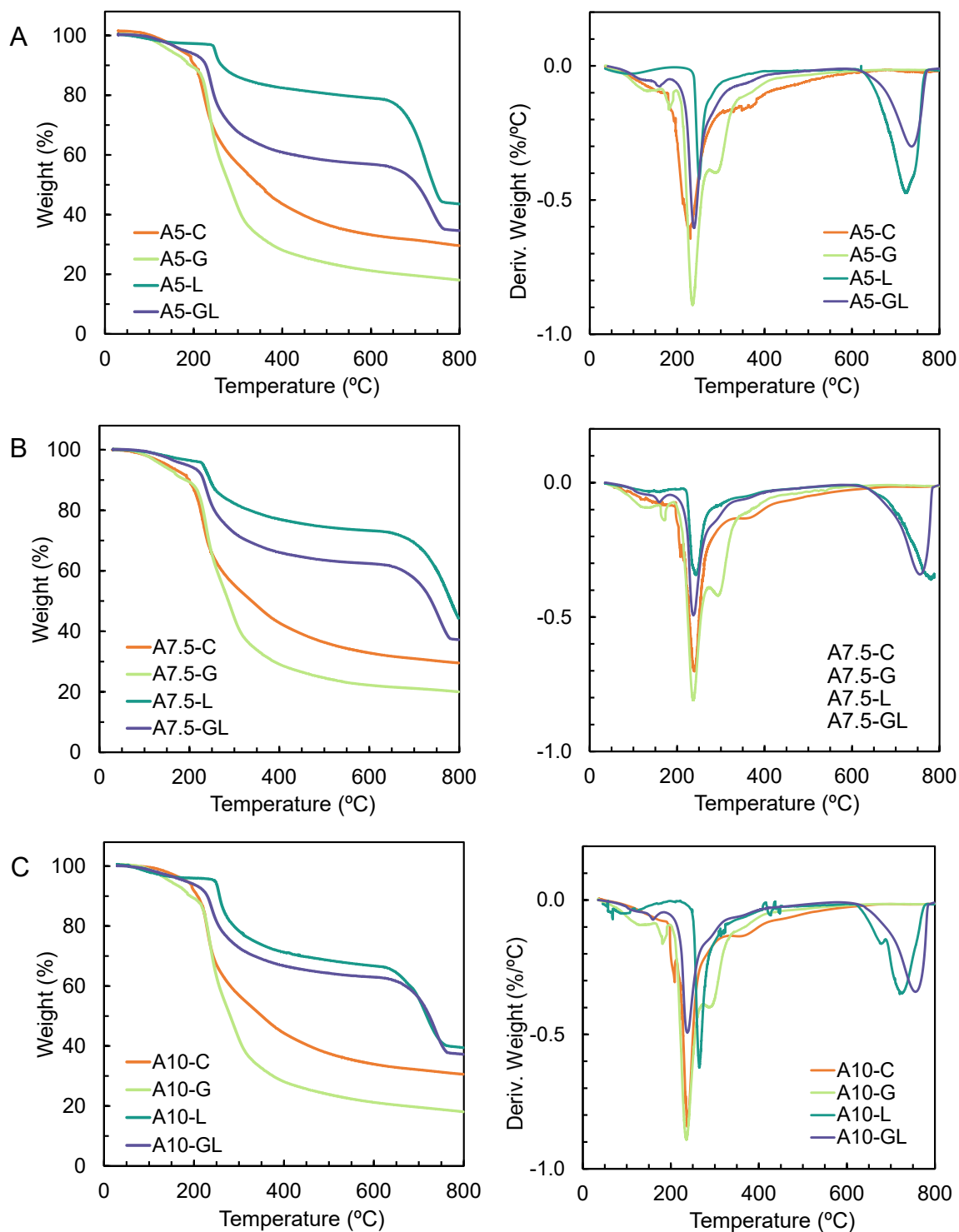


Figure 2.3: Representative results from thermogravimetric analysis for (A) 5% agar, (B) 7.5% agar, and (C) 10% agar specimens. Each sample was tested in duplicate.

TGA characterization enables direct comparison between traditional petroleum-based construction materials and agar-based materials. Commonly used plastics including polyethylene,

polypropylene, polystyrene, and polyethylene terephthalate begin to thermally degrade between 250 and 450 °C, with the maximum thermal degradation taking place between 420 and 490 °C (Miandad et al. 2019). Petroleum-based asphaltic binders for roadway or roofing applications begin to degrade between 300 to 350 °C (Bonati et al. 2012) and degradation continues until the temperature reaches 500 °C, where typically less than 20% of the original mass is retained (Ragab et al. 2016; Sá Araujo et al. 2014). Agar-based materials exhibit partial thermal degradation at lower temperatures than petroleum-based materials and binders commonly utilized in the construction industry (*i.e.*, beginning at 210 °C versus 250-300 °C). However, these data suggest that the addition of limestone filler can significantly improve the thermal stability of agar-based materials allowing for a tailorability of thermal durability. Previous work on asphalt mastics with additives including aluminum hydroxide, magnesium hydroxide, and limestone filler have shown similar improvements in thermal stability with the addition of a material that is inert until higher temperatures (Bonati et al. 2012).

Fungal Resistance

Photographs of a representative sample for each agar-based material formulation are displayed in Figure 2.4 and final ratings according to ASTM G21 ranging from 0 (no growth) to 4 (heavy growth) are shown in Table 2.4. The control agar samples exhibited good fungal resistance and only traces of fungal growth (*i.e.*, <10% of sample area) were observed after 28 days. The fungal resistance of samples increased with the addition of limestone and less sporulating growth was observed on limestone samples as compared to control counterparts. Most notably, the addition of glycerol significantly decreased fungal resistance. At 28 days, most samples without glycerol addition (*i.e.*, C and L samples) were rated as 1 (traces of growth). Contrastingly, the samples containing glycerol were rated in the range of 2 (light growth) to 4 (heavy growth). The experiment was validated using both a negative control (*i.e.*, no sample) and positive control (*i.e.*, cellulose). At 28 days, no growth was observed on the negative control specimens (rating of 0), and full growth coverage was observed on the positive control specimens (rating of 4).

Table 2.4: Final ASTM G21 ratings for triplicate samples of each agar-based material. Ratings were performed at the end of a 28-day incubation period at 30 °C.

Fungal Growth Rating												
	C			G			L			GL		
5% Agar	1	1	1	2	2	2	1	1	1	3	2	3
7.5% Agar	1	1	1	2	3	3	1	1	1	4	4	3
10% Agar	2	1	1	4	3	4	1	1	1	3	3	4

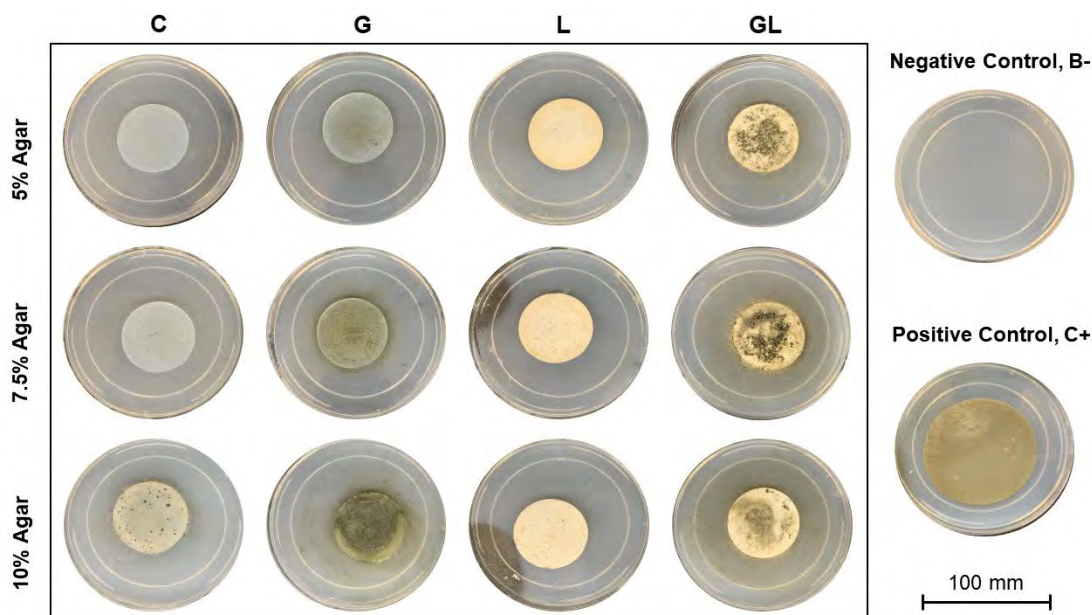


Figure 2.4: Representative photographs of agar-based materials subjected to ASTM G21 biodeterioration testing. All photographs were taken at the end of a 28-day incubation period at 30 °C.

In the current study, because only traces of growth were observed on the samples without glycerol 28 days after inoculation, it was concluded that agar and limestone did not serve as a primary carbon source for the growth of heterotrophic microorganisms, which is known to lead to biodeterioration. Agar is used as a microbiology culture media because it is non-nutritive for the vast majority of microorganisms making it a promising material candidate for construction application. However, glycerol provided an effective carbon source for fungal growth on samples. Similar results were obtained by Nissa *et al.* (2019), where 80% growth coverage (equivalent to ASTM G21 rating of 4) was observed on a starch-based bioplastic containing glycerol after just 10 days of incubation.

Microorganisms have evolved to use the hydrocarbons in petroleum as a source of carbon which can lead to biodegradation (Atlas and Hazen 2011). Microorganisms are typically introduced into crude oil and petroleum products during ventilation, pumping, and water flooding associated with oil extraction, transportation, and storage. The biodeterioration of petroleum products can worsen physical and chemical properties and lead to filter contamination or valve malfunctioning at refineries and reduced efficiency of petroleum products (Yemashova et al. 2007). To combat this, chemical biocides are often introduced. Notably, the chemical substances used to combat biodeterioration are often pollutive, mutagenic, and carcinogenic (Obahiagbon et al. 2014). Comparatively, the fungal resistance properties of agar-based materials would limit the use and necessity of harmful biocides.

Moisture Sorption

The equilibrated moisture content of agar-based binders, calculated using Equation 2.1, is shown below in Figure 2.5. In general, agar-based binders absorbed a large percentage of moisture relative to the conditioned mass of samples (*i.e.*, samples incubated at 30 °C until constant mass). This behavior was expected due to the large water content of agar-based binder structures and the hydrophilic behavior of hydrogels (Liu et al. 2021). After sample conditioning, agar-based binders retained 5 to 23% of fresh-state mass and upon immersion in distilled water, agar-based binders swelled to 55 to 97% of fresh state mass. The dimensional change associated with dehydration is illustrated in Figure 2.6. A decrease in rehydrated mass as compared to fresh state mass was expected. Entrapped excess water is present in hydrogels when initially prepared. When dehydrated, this entrapped water is removed and the ingress of excess water is opposed by the physical crosslinks of the agar structure (Lyons et al. 2009).

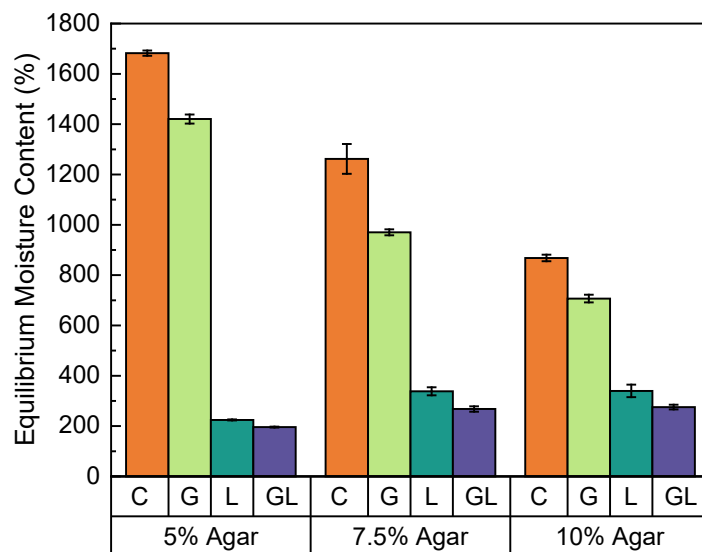


Figure 2.5: Equilibrium moisture content of agar-based materials immersed in 20 ± 2 °C distilled water. Error bars represent one standard deviation of triplicate measurements.

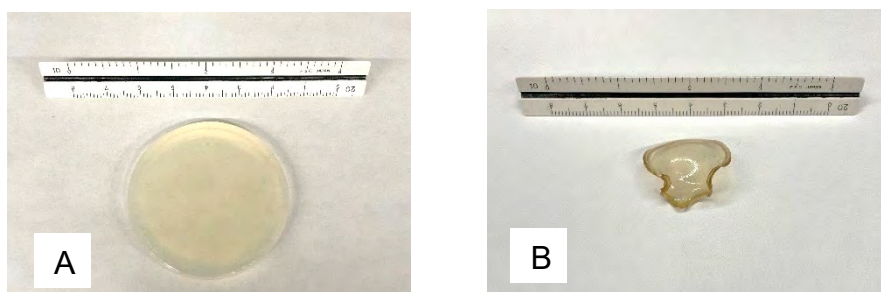


Figure 2.6: A5-C sample (A) initially prepared and (B) after dehydration to constant mass. Scale shown is 4 inches in length.

The equilibrated moisture content for control agar-based samples (see Figure 2.5) ranged from 196% to 1,682%, 268% to 1,262%, and 275% to 868% for A5, A7.5, and A10 samples, respectively. The maximum moisture content of control and glycerol dosed agar-based binders decreased with an increase in agar-concentration. These experimental observations are consistent with previous studies on the water holding capacity of agar hydrogels. The swelling behavior of agar is highly dependent on the porous size between crosslinked bonds. An increase in agar content results in a dense polymer network (*i.e.*, decreased porous space) which can hinder polymer mobility and decrease water holding capacity (Ghebremedhin et al. 2021; Pourjavadi et al. 2009; Rani et al. 2018).

The use of glycerol decreased water sorption for samples herein. Consistently, samples with glycerol absorbed less than comparative samples without glycerol (*i.e.*, G and GL compared

to C and L, respectively). Previous work conducted by Kusmono & Abdurrahim and Lavorgna et al. on chitosan films have noted similar decreases in water sorption with the use of glycerol (Kusmono and Abdurrahim 2019; Lavorgna et al. 2010). It is hypothesized that this behavior is due to the formation of bonds between biopolymers and glycerol, which increases the network density. Additionally, glycerol molecules take up intermolecular space which can decrease access to active sites for monomers to bond with water.

Notably, the inclusion of limestone significantly reduced the moisture sorption of agar-based materials. These findings align with literature on hydrogel-based composites. Rigid fillers can increase composite density and mechanical stability which can alter the swelling capacity of hydrogels (Dave and Gor 2018; Utech and Boccaccini 2016). Similar to glycerol findings, the decrease in moisture sorption with the use of limestone filler may be due to a sort of barrier effect. The limestone filler molecules can occupy intermolecular space and oppose moisture transport through the material.

Sample deterioration (*i.e.*, bath clouding, small sample fragments) was visible in testing of agar-based materials containing glycerol. This observation was validated by mass loss data obtained from re-conditioned sample masses using Equation 2.2 and shown in Figure 2.7. Agar-based binders with glycerol lost 19-22% more mass than samples without glycerol. Similar mass loss due to glycerol leaching has been noted in previous work on polymers (Jones et al. 2013; Wei et al. 2019). Interestingly, agar-based materials with both glycerol and limestone only lost 8-11% more mass than samples with only limestone. These results indicate that agar sample formulations with limestone may have a stronger and more dense gel network than formulations without limestone and may be able to withstand the stresses due to drying and shrinkage more successfully (Labropoulos et al. 2001). Conversely, sample formulations with glycerol may be less apt at withstanding drying and shrinkage.

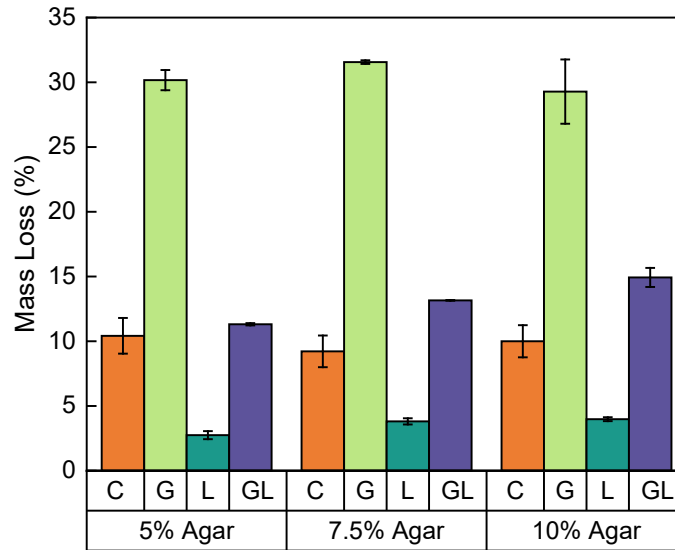


Figure 2.7: Mass loss of agar-based materials tested in moisture sorption in 20 ± 2 °C distilled water. Error bars represent one standard deviation of triplicate measurements.

As expected, these findings substantiate a propensity for agar-based material to exhibit high moisture sensitivity (i.e., high equilibrium moisture contents) and propensities for dimensional change and deterioration due to wetting and drying (i.e., mass loss). The moisture sorption characteristics highlight an inherent weakness associated with biopolymers, namely hydrophilicity. Moisture sorption behavior should be improved before large scale implementation of agar-based materials in construction. The moisture behavior of agar-based materials and methods to improve the hydrophobicity of agar-based materials are explored further in Chapter 3.

2.5 Evaluation as an Alternative Pavement Binder

Rotational Viscosity

The results of rotational viscosity (RV) measurements for the agar-based materials are displayed in Figure 2.8. The PG specification (ASTM International 2021a) dictates an RV threshold of < 3 Pa-s for asphalt binders, represented by a dashed line in Figure 2.8. The RV of A5, A7.5, and A10 samples ranged from 1.03 to 2.01 Pa-s, 8.08 to 10.64 Pa-s, and 23.16 to 32.23 Pa-s, respectively. All A5 agar-based materials passed RV standards set by the PG specification for asphaltic binders, while all A7.5 and A10 agar-based materials exceeded current RV standards. Typically, higher RV asphalt binders are used in warmer climatic regions and lower RV binders are used in colder climatic regions (Mallick and El-Korchi 2018b). Results obtained in this study suggest that agar-based materials, if used in flexible pavement applications, may be better suited for warm-climate

applications where a stiff material response is beneficial. However, workability of an asphalt mix is not merely a function of asphalt binder RV. Additional factors such as binder lubricity and the type, shape, texture, gradation, and porosity of aggregate in a mixture have been shown to greatly influence the workability of an asphalt mixture (Hanz and Bahia 2013; Mugume and Kakoto 2020).

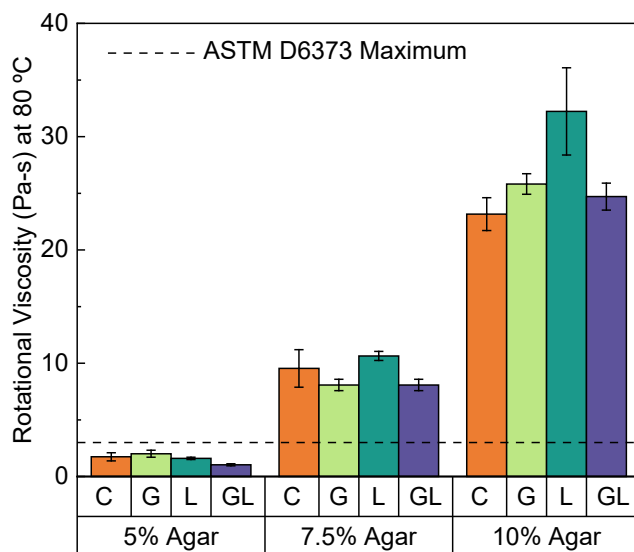


Figure 2.8: Rotational viscosities (RV) of agar-based materials with no additives (C), glycerol (G), and limestone (L). Error represents $\pm$ one standard deviation of triplicate measurements.

As expected, RV of agar-based materials increased as the agar concentration increased. Similar trends in increasing viscosity of higher-concentration agar were noted in work by Fernandez et al. (n.d.) and Zhenhua et al. (2020) using a parallel plate rheometer and a rotational viscometer, respectively. Notably, the inclusion of glycerol (40% w/w of agar) and limestone (15% w/w of agar) additives did not significantly impact the RV of agar-based binders. No significant trend in RV measurements was observed relative to the addition of each additive and the values of the glycerol- and limestone-containing samples are similar to their respective controls.

Glycerol is one of the most widely used plasticizers for biopolymers due to excellent compatibility with biopolymer chain structures (Arham et al. 2018). Glycerol molecules occupy intermolecular spaces between biopolymer chains, which in turn decreases attractive intermolecular forces and increases chain mobility (Gurgel Adeodato Vieira et al. 2011). However, it might be possible that a higher addition of glycerol is needed to observe a measurable plasticizing effect in the agar-based binder formulations investigated herein. For example, Yang and Paulson (2000) reported that the lowest effective glycerol concentration was 60% w/w for gellan films. As further discussed in the fungal resistance and moisture sorption results, higher amounts of glycerol,

however, would likely have a greater negative effect on the ability of agar-based materials to resist fungal biodeterioration and hydrolytic degradation, indicating a tradeoff in fresh-state workability performance and long-term durability.

The RV results suggest that application of agar-based materials would necessitate minor modifications to traditional placement protocol for asphalt binders. Typical hot mix asphalt is mixed at temperatures between 140 and 180 °C before being transported to a construction site, placed, and compacted (Thom 2014). The PG specification utilizes an RV testing temperature of 135 °C to mimic the conditions during construction. The lower temperature compatibility (*i.e.*, 80 °C) of agar-based materials indicates the possibility of mixing and compacting agar mixes (*i.e.*, agar-based binders and aggregate) at a lower temperature than typical hot mix asphalt. Implementation of lower placement temperatures corresponds to a reduction in material embodied energy as well as improved environmental safety. A mixing temperature reduction of 40 to 60 °C has been shown to reduce energy consumption of pavement construction by up to 40% (Kheradmand et al. 2014). Additionally, a lower placement temperature allows for quicker reopening of newly paved infrastructure to traffic and a longer paving season (Rubio et al. 2012).

Dynamic Shear Rheometry

Dynamic shear rheometry (DSR) was performed to determine the complex modulus (G^*) and phase angle (δ) for agar-based materials at temperatures ranging from 10 to 80 °C. These results are shown in Figure 2.9. Agar-based materials retained high stiffness (*i.e.*, $G^* > 7,000$ Pa) at test temperatures and the phase angle was consistently between 15° and 30° for the formulations and temperatures investigated herein. These results align with previous rheological studies on 1% and 1.5% agar and agar blends (Norziah et al. 2006; Somboon et al. 2014). Agar gels retain strength at high temperatures (*i.e.*, 70-80 °C) analyzed in this study because it is below the temperature necessary (*i.e.*, ~85 to 90 °C) for agar to attain random coil formation to significantly alter the tight 3-D gel network (Labropoulos et al. 2001).

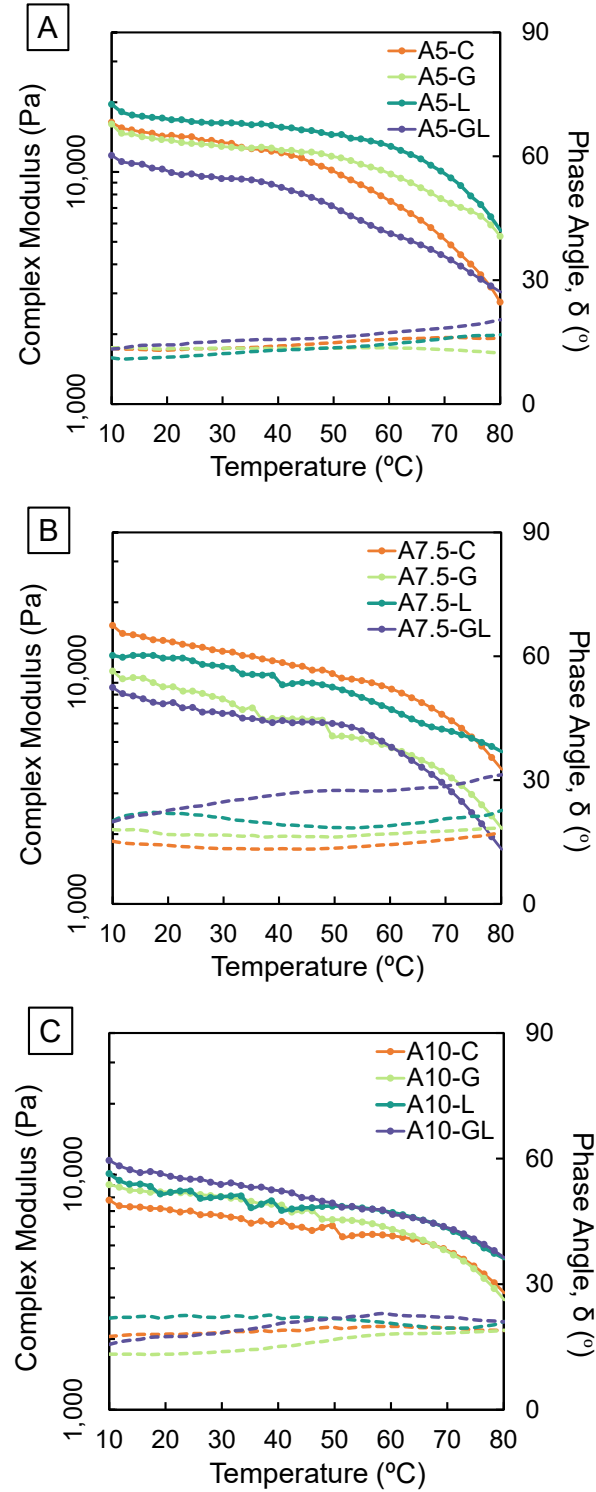


Figure 2.9: Representative results from dynamic shear rheometry (DSR) temperature sweeps for (A) 5% agar, (B) 7.5% agar, and (C) 10% agar specimens. Each sample was tested in duplicate.

In general, as the testing temperature increased, the complex moduli decreased, and the phase angle increased. Similar behavior has been observed in agar (Ghebremedhin et al. 2021) and

asphaltic binders, as noted in work conducted by Zhang et al. (2016), and Al-Khateeb et al. (2015). Compared to petroleum-based asphalt binders, agar-based materials exhibited a higher complex modulus and a lower phase angle at PG testing temperatures. The phase angle of petroleum-based asphalt binders is typically between 80 and 90 °C (Gao et al. 2020; Yan et al. 2022) indicating a highly viscous material response. The rheological response of agar-based materials corresponds to stiffer material behavior, which may indicate superior binder resistance to permanent deformation as compared to traditional binders.

Previous work with asphalt mastics has reported an increase in material complex moduli with the use of filler materials like fly ash, stone and brick dust, and mineral fillers (Huang and Zeng 2007; Naveed et al. 2019). In this work, no significant difference was observed relative to the addition of limestone filler. Similar to results obtained in rotational viscosity (RV) testing, no significant trend was observed relative to the addition of glycerol or limestone. DSR results for samples with glycerol validate the conclusions of RV data. A greater concentration of glycerol and limestone may be necessary to significantly impact the rheological properties of agar-based materials.

The rutting factor, calculated using Equation 2.3, is illustrated in Figure 2.10 for temperatures ranging from 10 to 80 °C. Isolated rutting factor results for 60 °C and 80 °C are shown in Figure 2.11. These temperatures were chosen as they closely resemble common upper pavement design temperatures for high temperature PG grading (*i.e.*, 58 °C and 82 °C). In the current study, the rutting factor calculated for agar-based binders at 60 °C and 80 °C ranged from 14.0 to 37.1 kPa and 6.9 to 15.6 kPa, respectively. These results suggest that all agar-based binder formulations possessed adequate stiffness to resist early-age rutting at upper pavement design temperatures less than 80 °C. Although these results point towards advantageous early-age rutting properties, the relatively high magnitude of complex moduli and rutting factors exhibited by agar-based binders may also indicate the possibility of low-temperature and fatigue-induced crack propagation. This is further investigated through bending beam rheometry testing of aged agar-based samples.

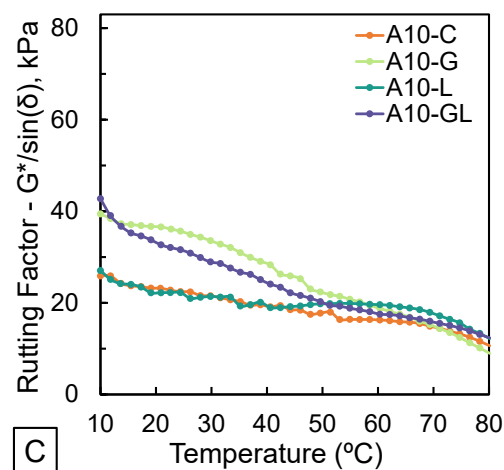
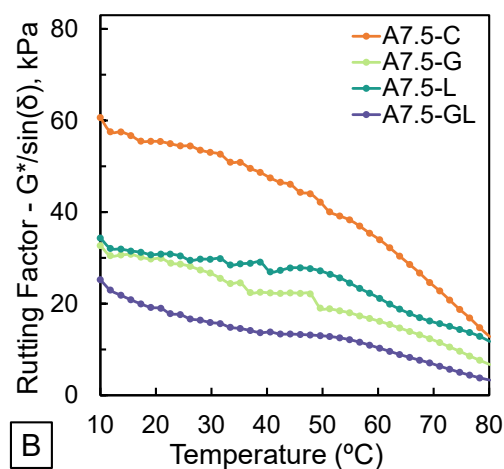
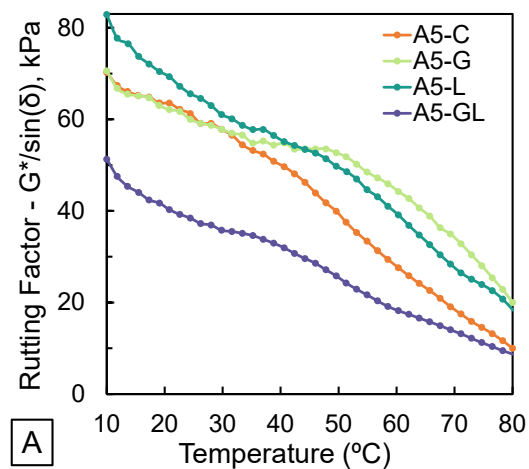


Figure 2.10: Representative results from dynamic shear rheometry (DSR) temperature sweeps illustrating the rutting factor for (A) 5% agar, (B) 7.5% agar, and (C) 10% agar specimens. Each sample was tested in duplicate.

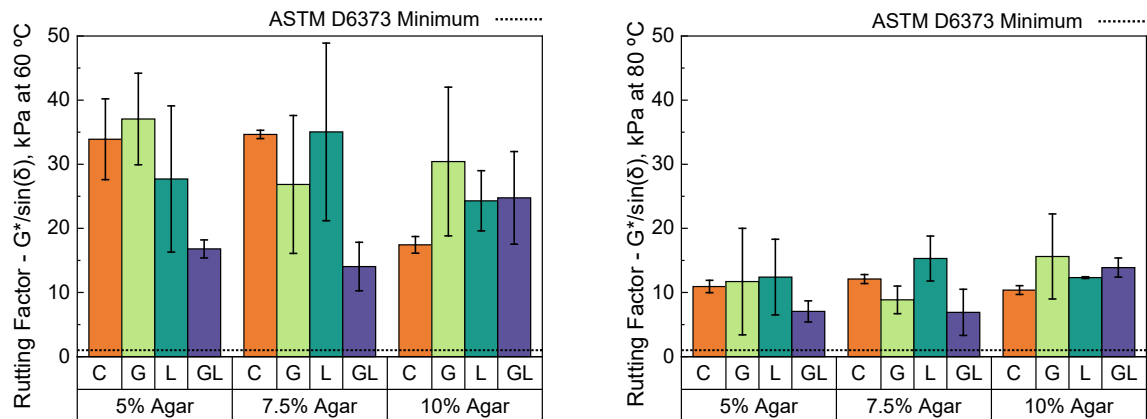


Figure 2.11: Mean rutting factor (*i.e.*, $G^*/\sin(\delta)$) for agar-binders at (A) 60 °C and (B) 80 °C. Error bars represent the high and low results for duplicate measurements.

Penetration

The results from penetration testing of agar-based materials binders are shown in Table 2.5. All four of the A5 sample formulations (*i.e.*, A5-C, A5-G, A5-L, A5-GL) and both A7.5 sample formulations without limestone (*i.e.*, A7.5-C, A7.5-G) exhibited a penetration greater than 350. Due to limitations based on the geometry of the penetration samples and apparatus, penetration measurements greater than 350 could not be accurately measured. The lowest penetration measurement, 162, was observed in both the A7.5-GL and A10-GL samples. Penetration measurements generally decreased as the agar concentration increased.

Table 2.5: Penetration results for agar-based binders. Error represents one standard deviation of triplicate measurements.

Penetration (0.1 mm)				
	C	G	L	GL
5% Agar	> 350	> 350	> 350	> 350
7.5% Agar	> 350	> 350	225 ± 8	162 ± 12
10% Agar	211 ± 12	217 ± 2	167 ± 3	162 ± 8

The addition of glycerol minimally impacted penetration measurements for 10% agar samples. The impact of glycerol was more pronounced in 7.5% agar samples with limestone (*i.e.*, A7.5-L as compared to A7.5-GL). The addition of limestone generally resulted in a decrease in penetration, particularly for the A7.5 and A10 samples. These results were expected as previous literature has reported a similar decrease in the penetration of asphalt binders with the use of fillers

like corn stalk fiber (Chen et al. 2019), crayfish shell powder (Lv et al. 2020), and rice husk ash (Han et al. 2017).

Traditional asphalt binders with high penetration values (*i.e.*, softer consistency) are typically used in cold climates to combat pavement cracking. Conversely, binders with low penetration (*i.e.*, stiffer consistency) are typically used in warmer climates where a binder is expected to resist permanent deformation at high temperatures. Asphalt binders with a penetration between 60 and 100 are mostly used in road construction, while asphalt binders with a penetration between 120 and 150 are primarily used in pavements with lighter traffic loading. Asphalt binders with penetration values between 200 and 300 are used less frequently for seal coating or arctic applications (Williams 1961).

Compared to traditional asphalt binders, agar-based materials exhibited relatively high penetration values. However, these results illustrate a spectrum of binder consistency that might be tailorable with agar concentration and the use of mineral fillers, like ground limestone. Additionally, these measurements were obtained under the most conservative conditions given that the agar samples were unaged and submerged in a 25 °C water bath at the time of testing. Just as petroleum-based binders are known to stiffen with age due to volatilization and oxidation (Mallick and El-Korchi 2018b), agar-based materials are expected to stiffen with age due to dehydration of the hydrogel structure at ambient conditions.

Bending Beam Rheometry

Bending beam rheometry (BBR) testing was conducted on a sub-set of the sample matrix. 10% agar-based binder samples (*i.e.*, A10) were not tested due to limited workability that led to inadequate mixing with Ottawa sand. Further, samples without limestone (*i.e.*, Control, C and Glycerol, G) were not tested in BBR due to dimensional instability, illustrated in Figure 2.12. Similar dimensional instability of agar hydrogels was noted in work conducted by Lyons et al. (2009). It was not possible to create homogenous samples with consistent geometry for the aforementioned agar sample types. Truncation of the sample matrix resulted in four sample types that were eligible for BBR testing: A5-L, A5-GL, A7.5-L, and A7.5-GL. Representative samples created for BBR testing by mixing agar-based binder with Ottawa sand are shown in Figure 2.13.

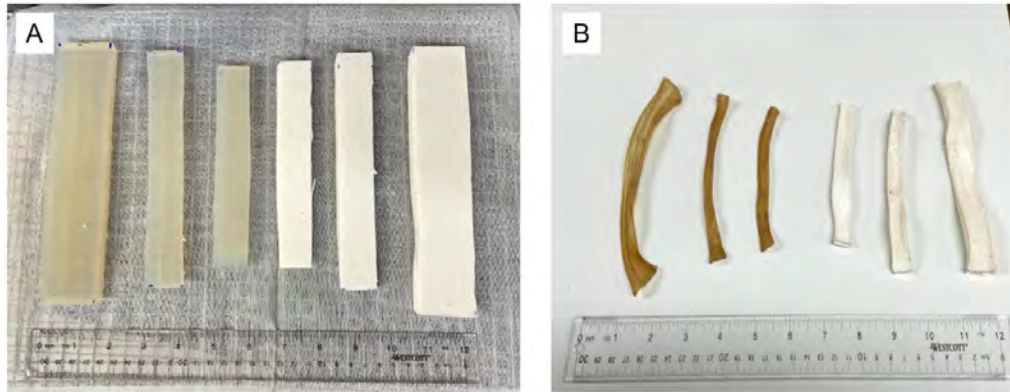


Figure 2.12: Dimensional change of A5-C (left) and A5-L (right) agar-based binder samples (A) freshly gelled and (B) dehydrated to constant mass.



Figure 2.13: Samples created for BBR testing by mixing agar-based binders with Ottawa sand at a ratio of 2:1. Representative specimen for A5-L agar-based binders measuring 0.5 in wide and 6 in long.

Representative deflection curves from BBR testing are shown in Figure 2.14. The deflection of agar-based samples was within testing range for ASTM D6648 (*i.e.*, deflection greater than 0.08 mm and less than 4 mm). The addition of glycerol led to a significant increase in the deflection of 5% agar samples but had a minimal impact on the deflection of 7.5% agar samples. These results support RV findings that a higher addition of glycerol is likely needed to observe a measurable mechanical impact in the higher concentration (*i.e.*, 7.5% and 10%) agar-based material formulations.

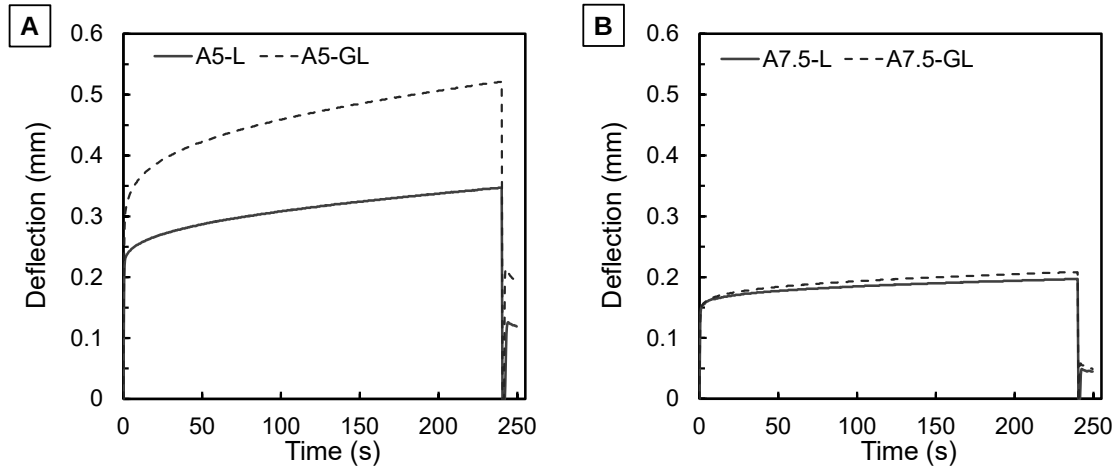


Figure 2.14: Representative deflection curves of Bending Beam Rheology testing at -12 °C for (A) 5% agar and (B) 7.5% agar. Each sample was tested in triplicate.

The average deflection, stiffness, and m-value for each agar-based binder tested is shown in Table 2.6. While the inclusion of glycerol in 5% agar samples did increase sample deflection and decrease sample stiffness, it did not significantly impact the m-value. Interestingly, the inclusion of glycerol in 7.5% agar samples did not significantly impact the deflection, stiffness, or m-value and A7.5-L and A7.5-GL samples behaved relatively similarly in BBR testing. None of the agar-based binders in this study passed m-value thresholds dictated by PG specification (*i.e.*, $m\text{-value} > 0.300$) indicating that these materials as currently designed may be unable to relax accumulated pavement stresses. While 5% agar samples did meet stiffness specifications (*i.e.*, $\text{Stiffness} < 300 \text{ MPa}$), 7.5% agar samples surpassed this specification. UV-irradiation can alter the molecular weight and photochemical stability of polysaccharides (Sionkowska 2014; Wang et al. 2005) and additional testing temperatures and aging mechanisms like UV exposure temperatures should be considered in future BBR testing of agar-based binders.

Table 2.6: Average deflection, stiffness, and m-value for agar-based binders at -12 °C. Error represents one standard deviation of triplicate measurements.

Bending Beam Rheometry Results			
Sample	Deflection (mm)	Stiffness (MPa)	m-value
A5-L	0.299 ± 0.019	269 ± 18	0.111 ± 0.008
A5-GL	0.435 ± 0.082	192 ± 38	0.118 ± 0.006
A7.5-L	0.194 ± 0.032	428 ± 64	0.057 ± 0.013
A7.5-GL	0.178 ± 0.015	457 ± 40	0.071 ± 0.003

2.6 Conclusions

This study evaluated the properties and durability of agar-based binders as well as explored the application of agar-based binders as a direct alternative material for traditional petroleum-based asphalt binders. The thermogravimetric, fungal, isothermal water sorption, and standard asphalt engineering properties (*i.e.*, rotational viscosity, dynamic shear rheometry, penetration, and bending beam rheometry) of 5, 7.5, and 10% w/w agar-based binders with and without glycerol and limestone additives were characterized and compared to traditional civil engineering materials.

The experimental data indicated the following conclusions regarding the durability of agar-based materials:

- Thermogravimetric analysis illustrated that all agar-based binders were thermally stable up to 210°C. The onset of thermal decomposition occurs at a slightly lower temperature for agar-based binders as compared to petroleum-based materials (*i.e.*, 210 °C vs. 250-300 °C).
- When subjected to ASTM G21 testing, agar-based binders without glycerol addition exhibited improved resistance to biodeterioration as compared to a positive control (*i.e.*, cellulose). Samples with glycerol addition showed substantial growth, indicating that glycerol served as an effective carbon source for growth of heterotrophic microorganisms.
- Moisture diffused rapidly in agar-based binders and the equilibrated moisture content for agar-based samples relative to conditioned mass ranged from 196% to 1,682%. Relative to fresh-state sample mass, rehydrated agar-based binders swelled to 55 to 97% moisture content. Limestone significantly reduced moisture sorption and improved mass loss during testing. However, glycerol plasticizer was found to leach from agar-based binder samples in isothermal sorption testing.

Generally, durability findings indicated that glycerol inclusion can reduce the thermal, biodeterioration, and moisture durability characteristics of agar-based materials. The addition of glycerol decreased the mass retained by agar-based materials at high temperatures, acted as a carbon source for biodeterioration, and increased the mass loss associated with drying shrinkage in moisture sorption testing. Conversely, findings indicated that limestone filler improved the durability characteristics of agar-based materials. The addition of limestone led to a higher percentage of retained mass at high temperatures, did not serve as a carbon source for

biodeterioration, and reduced the moisture sorption and mass loss associated with immersion testing.

In work evaluating the engineering characteristics of agar-based materials in pavement applications, the experimental data indicated the following conclusions:

- All 5% w/w agar-based binders passed the rotational viscosity (RV) threshold set by the performance grade (PG) specification ($< 3 \text{ Pa}\cdot\text{s}$), while all 7.5% and 10% w/w samples exceeded that same threshold. In general, agar-based binders exhibited more viscous behavior than traditional asphalt binders. However, RV testing of agar-based binders was completed at a lower temperature than stipulated by the PG specification (*i.e.*, 80°C vs. 135°C). The lower temperature compatibility of agar-based materials indicates the possibility of lower mixing and compaction temperatures and an increase in energy efficiency of production as compared to petroleum-based binder production.
- Dynamic shear rheometry (DSR) revealed that all agar-based binders in this study exhibited adequate stiffness to resist early-age rutting at temperatures up to 80°C . Agar-based materials generally illustrated a higher complex modulus and lower phase angle than traditional asphalt binders, which is advantageous for rutting. Relatedly, the high magnitude of the complex moduli at testing temperatures might indicate the possibility of low-temperature and fatigue-induced cracking if these materials were used as designed in pavement applications.
- Penetration measurements performed on fully submerged, unaged agar-based binders ranged from 162 to greater than 350. While penetration readings were generally higher than those associated with traditional asphalt binders used in road applications, the results indicated that penetration consistency might be tailorable with the use of additives, such as ground limestone. Furthermore, penetration of agar-based binders is expected to further decrease with aging.
- None of the agar-based binders tested in bending beam rheometry (BBR) testing passed m -value thresholds dictated by PG specification (*i.e.*, $m\text{-value} > 0.300$) which may indicate that these materials as currently designed will be unable to relax accumulated pavement stresses. While 5% agar samples did meet stiffness specifications (*i.e.*, Stiffness $< 300 \text{ MPa}$), 7.5% agar samples surpassed this specification.

Notably, the inclusion of limestone and glycerol did not significantly impact the rheological properties (*i.e.*, rotational viscosity and dynamic shear rheometry) of samples. It is hypothesized that a higher dose of these additives is needed to see measurable differences in the rheological properties of agar-based materials. Although increasing the dosage of glycerol used in agar-based binder formulations may improve rheological properties, a greater dosage would come with a trade-off in biodeterioration, thermal, and moisture sorption properties.

Asphalt binders were chosen as a reference material and investigated as a potential application for agar-based binders due to synergistic behavior and properties (*i.e.*, viscoelasticity, temperature needs). Traditional asphalt binder testing methods were utilized in order to communicate novel agar applications with an established technical field (*i.e.*, pavement engineering). Results herein illustrate that agar-based materials are more stiff than traditional asphalt binder materials, which may be problematic in regard to fatigue and low temperature stresses that accumulate in pavement materials. The rigid characteristics of agar-based materials indicate that these materials may perform adequately in regard to rutting, but they do not have the same ability as traditional petroleum-based asphalt binders to relax accumulated stresses.

Although this work highlights the benefits of agar-based material use (*i.e.*, lower temperature requirements, advantageous rutting characteristics, strong biodeterioration resistance), portions of this work highlight inherent weaknesses associated with agar-based materials. Namely, the dimensional change associated with agar-based materials as noted in moisture sorption and BBR testing. The moisture sorption properties of agar-based binders should be acknowledged and improved upon before implementation of agar-based binders. This behavior could be improved using physical (*i.e.*, coatings, filler materials) or chemical (*i.e.*, chemical crosslinking) modification methods. Functional improvements in these moisture related properties are further investigated in Chapter 3.

Application specific results from this work indicate that agar-based materials are likely not appropriate for replacement of surface course asphalt binders. The moisture sensitivity, dimensional instability, and relative rigidity of agar-based materials may make it a poor candidate for use as a surface pavement structure. The use of agar-based materials in the layers under a pavement surface course (*i.e.*, base or subbase course) may be a more appropriate application as these layers are designed to be rigid and less exposed to surface moisture (*i.e.*, they are protected by the surface course material). This range of applications is further investigated in Chapter 4.

3. Characterization and Modeling of the Moisture Diffusion of Agar-Based Materials

3.1 Introduction

Biopolymers are potential sustainable substitutes for petroleum-derived materials due to their renewability, reduced carbon footprint, and biodegradability at the end of their lifespan. However, the hydrophilicity of biopolymers can impede their widespread use in industrial applications and degrade the material appearance, stability, durability, biodeterioration, and mechanical properties (Billington et al. 2014). The moisture sorption properties and long-term performance of some biopolymers are a barrier to their widespread implementation and many features and characteristics regarding moisture sorption remain to be identified to facilitate their largescale implementation.

Agar, a biopolymer extracted from red seaweed illustrates many synergistic traits with typical civil engineering materials (*i.e.*, plastics, thermoplastic asphalt binders). Agar gels are highly hydrophilic and can absorb as much as 1,600% relative to their dehydrated mass (Nasef et al. 2011). Previous work in this project (*i.e.*, Chapter 2) has indicated a high propensity for moisture sorption and swelling of agar. This propensity for moisture sorption can create voids and weaken interfacial adhesion in materials. As such, this behavior requires more investigation on physical and chemical modification methods for tailoring and controlling this hydrophilic behavior (Andreopoulos and Tarantili 2002).

The duration and intensity of moisture diffusion and transport is influenced by polymer and permeant structures and solvent-polymer interaction. Introduction of moisture causes structural changes of polymer from glassy to rubbery state. In order to combat this, significant work has been conducted to produce models to describe mass transport. Although there are many models to describe mass transport processes, Fick's laws are often applied due to their simplicity and mathematical tractability (De Kee et al. 2008).

Fick's laws have been used in previous literature to analyze transparent wood composites (Foster et al. 2021), PHBV wood flour composites (Srubar et al. 2012), and gelatin adhesives (Hess and Srubar 2016). To authors' knowledge, no work has focused on highly concentrated agar gels. Further, very little of this work has focused on improving properties for pavement applications. In order to overcome these limitations, researchers and engineers have investigated methods of

enhancing biopolymers including both physical and chemical modifications. Understanding and addressing moisture sorption properties of biopolymers is a crucial step in expanding their use to allow for an extended range of applications.

3.2 Research Objectives

The primary goal of this chapter is to characterize moisture sensitivity recognized in previous work (i.e., Chapter 2) and identify modification methods to improve moisture durability of agar-based binders. In order to investigate functional improvements in the hydrophilicity of agar-based materials, three subsets of materials were investigated: (1) unmodified (i.e., pure agar control samples at three concentrations), (2) physically modified (i.e., dosed with an inert limestone filler or coated in a protective polyurethane coating), and (3) chemically modified (i.e., crosslinked with maleic anhydride) samples. Fourier Transform Infrared (FTIR) spectroscopy was used to verify the modifications. Moisture sorption experiments were used to compare the efficacy of treatments. Additionally, experimentally obtained data was used to classify the diffusion behavior of agar-based materials and calculate water transport parameters to produce empirical models of diffusion behavior.

3.3 Materials and Methods

Materials

Agar, maleic anhydride, and sodium hydroxide powder were supplied by Sigma Aldrich (St. Louis, MO, USA). Limestone powder (calcium carbonate, CaCO_3) with a particle size $<250\ \mu\text{m}$ and polyurethane coatings were supplied by Research Products International (Mt Prospect, IL, USA) and Republic Powdered Metals, Inc (Medina, OH, USA), respectively.

Methods

Sample Preparation

Unmodified control samples (i.e., agar hydrogels without physical or chemical modification) were prepared at 5, 7.5, and 10% w/w agar concentration (i.e., relative to deionized water). Two additional subsets of 5% w/w agar samples were evaluated to investigate the impact of physical and chemical modifications on the moisture sorption characteristics of agar. The physical modification methods investigated include the use of an inert filler material (i.e., 15% w/w limestone powder) or a protective physical coating (i.e., either water or oil-based polyurethane). Physical coatings were chosen for evaluation based on their ease of use, durability, and effective

chemical and water resistance. Chemically modified agar samples were crosslinked with maleic anhydride at four concentrations (*i.e.*, 1%, 5%, 10%, and 30% w/w relative to agar). Sample formulations and naming convention are summarized in Table 3.1.

Table 3.1: Sample formulations and naming convention.

Sample Subset	Sample Designation	DI Water (mL)	Agar (g)	Limestone (g)	Polyurethane Coating	Maleic Anhydride (g)
Unmodified (Control)	A5	200	10	-	-	-
	A7.5	200	15	-	-	-
	A10	200	20	-	-	-
Physically Modified	L	200	10	30	-	-
	OB	200	10	-	Oil-Based	-
	WB	200	10	-	Water-Based	-
Chemically Modified	1% MA	200	10	-	-	0.1
	5% MA	200	10	-	-	0.5
	10% MA	200	10	-	-	1.0
	30% MA	200	10	-	-	3.0

In order to prepare unmodified agar samples, 200-mL of deionized water was added to 500-mL media bottles and the appropriate amount of agar powder (*i.e.*, 10, 15, or 20-g) was dissolved at ambient laboratory conditions (22 ± 2 °C) under continuous agitation using a magnetic stir bar. The media bottles were placed in a Yamato autoclave (Santa Clara, CA, USA) and heated to 100 °C for 20-min. Agar samples physically modified with limestone filler were prepared similarly, but the appropriate amount of ground limestone filler (*i.e.*, 30-g) was mixed into fresh gels by hand for approximately 1-min prior to sample molding.

Chemically modified agar samples were cross-linked with 1%, 5%, 10%, and 30% maleic anhydride (*i.e.*, relative to the agar concentration) using methods outlined in work conducted by Ye et al. (2022). The appropriate amount of maleic anhydride powder (*i.e.*, 0.1, 0.5, 1, or 3-g) was dissolved in 200-mL of 5% w/w agar solution at ambient laboratory conditions under constant agitation with a magnetic stir bar. The pH of solutions was adjusted to 7.0 using a 2.0 M NaOH solution. Solutions were then heated to 70 °C for 30-min on a Corning hot plate before sample molding.

Agar gels were poured into 55-mm diameter molds to create disc- shaped samples with a depth of approximately 10-mm. After gelling for 30-min at ambient laboratory conditions (22 ± 2 °C), samples were removed from molds and weighed to determine the fresh sample mass. Samples

were then conditioned in incubation at 30 °C until reaching constant mass, defined as < 1% change in mass in a 24-hr period. Samples modified with the use of a physical coating system were prepared identically to unmodified samples and coated in three layers of oil-based (*i.e.*, OB) or water-based (*i.e.*, WB) polyurethane after sample conditioning. Representative samples after coating are shown in Figure 3.1.



Figure 3.1: 5% w/w agar sample coated in water-based (left) or oil-based (right) polyurethane.

Fourier Transform Infrared Testing

Agar samples were examined through attenuated total reflectance (ATR) Fourier transform infrared (FTIR) testing using a ThermoScientific Nicolet iS20 (Fisher Scientific). FTIR was used to identify peaks associated with sample modification methods (*i.e.*, identify new peaks associated with the use of an inert filler or crosslinking with maleic anhydride). FTIR samples were conditioned to constant mass and then crushed to produce powders for each sample. The spectra of three samples were collected and each spectrum was an average of 32 measurements scanned from 600 to 4,000 cm^{-1} at a resolution of 1 cm^{-1} .

Moisture Sorption Characteristics

The moisture sorption properties were determined according to a modified ASTM D570 procedure where the conditioning temperature was lowered from 50 °C to 30 °C (ASTM International n.d.). After conditioning to constant mass, agar samples were immersed in 20 ± 2 °C deionized (DI) water. At set testing intervals, samples were removed from the DI water bath, the surface moisture was dried, and the mass of each sample was recorded. Sample masses were recorded after 0.25, 0.5, and 0.75 hrs of immersion and every hour thereafter for the first eight hours of immersion. Sample masses were then recorded approximately every 24 hrs until reaching equilibrium (*i.e.*, < 1% change in mass in a 24 hr period). The intermittent moisture content (M), was calculated for each increment of immersion time using Equation 3.1:

$$M (\%) = \frac{m_t - m_c}{m_c} * 100\% \quad \text{Equation 3.1}$$

where m_c and m_t are the sample mass after initial conditioning to constant mass and at a given time of immersion, respectively. The moisture content was plotted against the square root of time to create characteristic moisture sorption curves. After reaching constant mass in immersed conditions, samples were reconditioned in an incubator at 30 °C until reaching constant mass and then cooled for approximately 1 hr and reweighed. The percentage of matter lost (M_{Loss}), during immersion testing was calculated for each sample using Equation 3.2:

$$M_{Loss} (\%) = \frac{m_c - m_{rc}}{m_c} * 100\% \quad \text{Equation 3.2}$$

where m_{rc} is the sample mass after reconditioning. Each agar sample type was tested in triplicate.

Classification and Modeling of Diffusion Behavior

Understanding and addressing moisture sorption properties of biopolymers is a crucial step in expanding their use for an extended range of applications. Water transport and diffusion can be classified from the experimental sorption curve using the following time-based power law relationship (Alfrey Jr. et al. 1966):

$$\frac{M_t}{M_{max}} = kt^n \quad \text{Equation 3.3}$$

where M_t is the moisture content at a given time and M_{max} is the equilibrated moisture content achieved while immersed. The constant k is a characteristic of the solvent-polymer system and the exponent, n , is a kinetic rate constant that can be used to describe the physical mechanism and general behavior of diffusion. Fickian, anomalous, and relaxation-controlled diffusion behavior can be classified according to $n \approx 0.5$, $0.5 < n < 1$, and $n \approx 1$ respectively.

Fickian diffusion (*i.e.*, $n=0.5$) indicates that the polymer relaxation occurs more quickly than the moisture diffusion (Foster et al. 2021; Yang et al. 2020). In this case, the polymer chains are flexible and immersion results in an instantaneous moisture sorption response (Islam et al. 2010). Relaxation controlled diffusion (*i.e.*, $n = 1$) indicates the opposite: the rate of moisture diffusion is faster than the relaxation rate of the polymer, resulting in a moisture boundary that advances towards the core of a material. Lastly, anomalous diffusion (*i.e.*, $0.5 < n < 1$) indicates a condition between Fickian and polymer relaxation controlled where moisture diffusion and

polymer relaxation are comparable (Foster et al. 2021; Ganji et al. 2010; Hess and Srubar 2016; Yang et al. 2020). Constants can be found by rearranging Equation 3.3 to yield:

$$\log \frac{M_t}{M_{max}} = \log k + n \log t \quad \text{Equation 3.4}$$

The constants k and n can be obtained from calculating the slope and intercept of $\log(\frac{M_t}{M_{Max}})$ vs $\log(t)$. Equation 3.3 does not accurately predict anomalous diffusion for values of $\frac{M_t}{M_{Max}} \geq 0.6$ (Ganji et al. 2010; Hess and Srubar 2016). In order to account for this, the first order Berens-Hopfenberg differential equation is proposed and can be integrated to obtain (Berens and Hopfenberg 1978):

$$\frac{M_t}{M_{max}} = 1 - Ae^{-k_2 t} \quad \text{Equation 3.5}$$

where k_2 represents the relaxation rate constant of the polymer network and A represents a constant. Equation 3.5 can be rearranged to obtain:

$$\ln(1 - \frac{M_t}{M_{max}}) = \ln A - k_2 t \quad \text{Equation 3-1}$$

Values of k_2 and n can be obtained by calculating the slope and intercept of $\ln(1 - \frac{M_t}{M_{Max}})$ vs time for the interval of moisture sorption data in which $\frac{M_t}{M_{Max}} < 0.6$ (Ganji et al. 2010; Hess and Srubar 2016; Kim et al. 2003). Experimentally obtained kinetic rate constants (*i.e.*, k , n , k_2 , and A) were used to define numerical diffusion models for agar materials and model results were compared directly to experimentally obtained sorption data. Results were used to characterize how modification methods changed water behavior of samples and provide a baseline model for practical engineering use.

3.4 Results and Discussion

Fourier Transform Infrared Testing

Fourier Transform Infrared (FTIR) spectroscopy was used to verify modification methods of agar. While crosslinking with maleic anhydride produces definitive chemical changes within the agar structure, physical modification methods do not alter the chemical composition of agar. Therefore, FTIR results can be used to illustrate the difference between chemical and physical modification methods.

The hydrophilicity of agar is due to four -OH groups present on the chemical structure that can readily react with water and form hydrogen bonds. Chemical cross-linking using maleic

anhydride was completed on 5% w/w agar in order to decrease the number of available hydroxyl groups. When introduced into agar solution, maleic anhydride forms two carboxylic acid groups (*i.e.*, R-C(=O)-OH). One of these groups is neutralized by sodium hydroxide (*i.e.*, used to pH solution) while the remaining carboxylic acid group reacts with the -OH group of agar to form an ester (*i.e.*, R-C(=O)-O-Na). Chemical reaction with maleic anhydride can decrease the number of hydroxyl groups available on the agar structure, which can reduce water attraction and subsequent bonding with water (Ye et al. 2022).

Ground limestone acts as an inert filler occupying space within the intermolecular structure and produces a denser material network. Filler materials can decrease the porous intermolecular space, hinder water mobility, and decrease water holding capacity (Ghebremedhin et al. 2021; Pourjavadi et al. 2009; Rani et al. 2018). Similarly, polyurethane coatings merely provide a protective water-resistant barrier for samples and act as a moisture shield, minimizing pathways in which moisture can enter a material.

Sample A5 in Figure 3.2 illustrates characteristic FTIR bands associated with unmodified agar. The peak visible at $1,055\text{ cm}^{-1}$ is common to polysaccharides and associated with C-C or C-O stretching (Vuai 2022). This band is related to the connection of galactose units within the agaropectin portion of agar that contributes to gel formation and strength (Guerrero et al. 2014). The absorption band at $1,430\text{ cm}^{-1}$ is associated with the vibration of C-H due to methyl (CH_3) or methylene (CH_2) groups (Vuai 2022) and the peak visible at $2,900\text{ cm}^{-1}$ is also attributed to methoxyl groups and related to C-H stretching (Guerrero et al. 2014). The broad band between $3,200$ and $3,500\text{ cm}^{-1}$ is associated with O-H stretching of hydroxyl groups (El-hefian et al. 2012; Pereira et al. 2003).

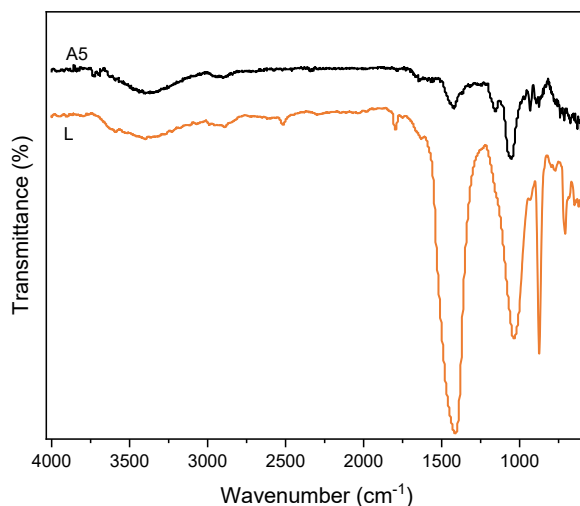


Figure 3.2: Representative FTIR spectra of unmodified 5% w/w agar (A5) and physically modified agar dosed with an inert limestone filler (L)

A representative spectrum for samples physically modified with limestone filler (*i.e.*, L) is shown in Figure 3.2 with the spectra of 5% w/w agar sample (*i.e.*, A5) acting as comparable control. Pure limestone (*i.e.*, CaCO_3) produces absorption peaks at wavelengths of 1,420 to 1,450 cm^{-1} , 860 to 890 cm^{-1} , and 710 to 725 cm^{-1} due to the carbonate (*i.e.*, CaCO_3) group (Al Omari et al. 2016; National Institute of Standards and Technology n.d.; Santos et al. 2021). Spectra for A5 samples physically modified with limestone filler illustrate the characteristic peaks of both unmodified agar and of limestone. The absence of independent, new peaks indicates chemical similarity and notably, the band related to O-H groups (*i.e.*, 3,200-3,500 cm^{-1}) remains unaffected with physical modification.

The spectra of pure maleic anhydride and of chemically modified samples are shown in Figure 3.3. Maleic anhydride (MA) crosslinked samples illustrate a characteristic peak formation at 1,580 cm^{-1} and the intensity of this peak increases with an increase in MA content. This peak is due to the stretching of carboxylic anions associated with the formation of esters. Additional evidence of chemical differences can be seen in bands associated with O-H groups. MA crosslinked samples illustrate a significant reduction in intensity of bands associated with O-H groups at 3,200-3,550 cm^{-1} , indicating that chemical modification with MA decreased the number of available hydroxyl groups on the structure of agar, as intended. (Ye et al. 2022). Similar differences in FTIR spectra were noted in work with MA crosslinked agar completed by Ye et al.

(2022). Overall, these results indicate that samples crosslinked with MA are chemically different and verify chemical treatment efficacy.

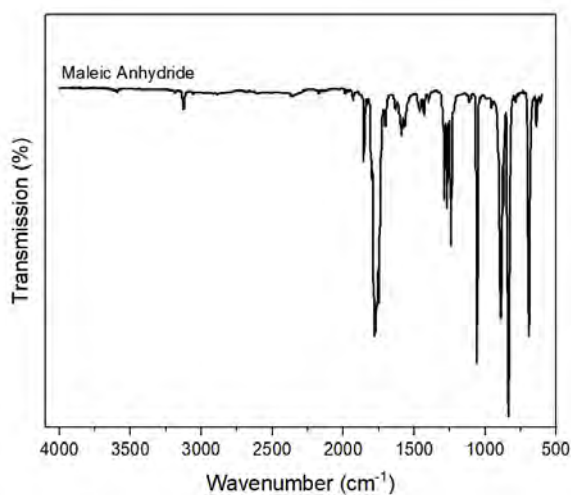


Figure 3.3: Representative FTIR spectra of pure maleic anhydride powder.

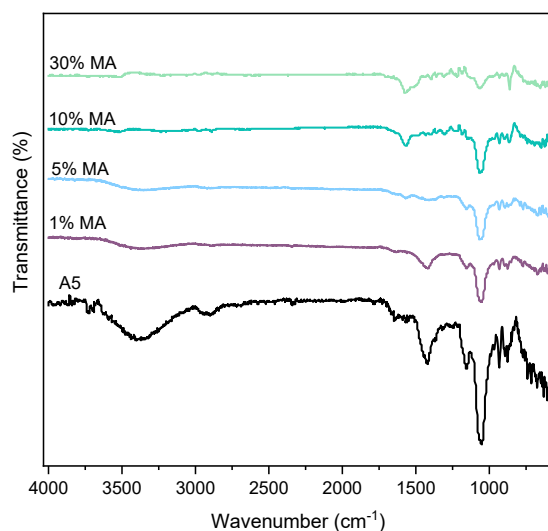


Figure 3.4: Representative FTIR spectra of unmodified 5% w/w agar (A5) and chemically modified agar crosslinked with maleic anhydride (MA).

Moisture Sorption Characteristics

The experimental sorption curves of 5, 7.5 and 10% w/w unmodified agar are shown below in Figure 3.5. The moisture content of samples was calculated using Equation 3.1 and plotted against the square root of time ($\sqrt{t}$). Upon immersion, the initial rate of moisture sorption is very high due to the filling of capillary spaces within the hydrogel structure. As free space within the hydrogel structure is filled with solvent, hydrogen bonds form between water and the -OH groups

present in agar. Water molecules begin to displace the polymer chain and the hydrogel structure is able to swell (Yang and Paulson 2000). In general, unmodified agar samples absorbed a large percentage of moisture relative to the conditioned mass of samples (*i.e.*, samples incubated at 30 °C until constant mass). This behavior was expected due to the chemical structure and hydrophilic behavior of agar (Liu et al. 2021) in addition to preliminary moisture sorption results obtained in Chapter 2.

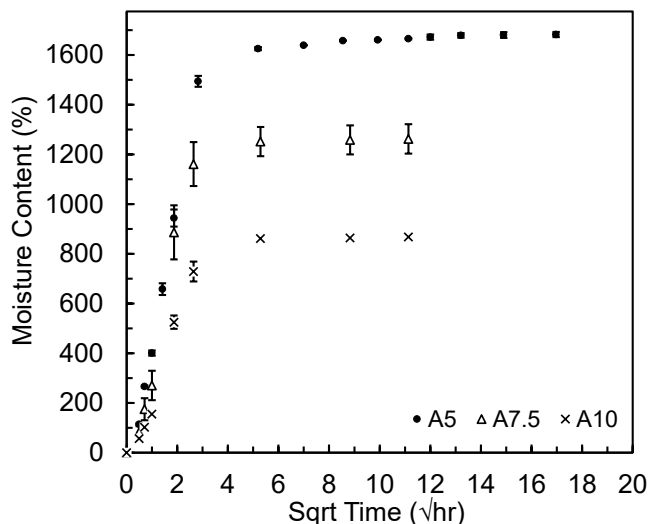


Figure 3.5: Characteristic sorption curves of 5, 7.5, and 10% w/w agar at 22 °C. Error bars represent one standard deviation of triplicate measurements.

The experimental moisture sorption curves of physically modified samples (*i.e.*, dosed with inert limestone or coated in a protective polyurethane) are shown in Figure 3.6. Oil-based (OB) and water-based (WB) polyurethane coated samples initially performed well upon immersion, absorbing virtually no moisture. After approximately 3-5 hours of immersion, imperfections in the polyurethane coating formed around sample edges, which initiated the ingress of moisture and eventual swelling of the sample beneath the coating. Imperfections and observed coating failure are shown in Figure 3.7. Polyurethane is susceptible to crack formation on high stress application areas which can lead to hydrolytic degradation (Sastri 2010). Notably, the water-based polyurethane samples (WB) failed before the oil-based polyurethane samples (OB) (*i.e.*, after 3 and 8 hours, respectively), indicating higher hydrolytic durability of oil-based polyurethane.

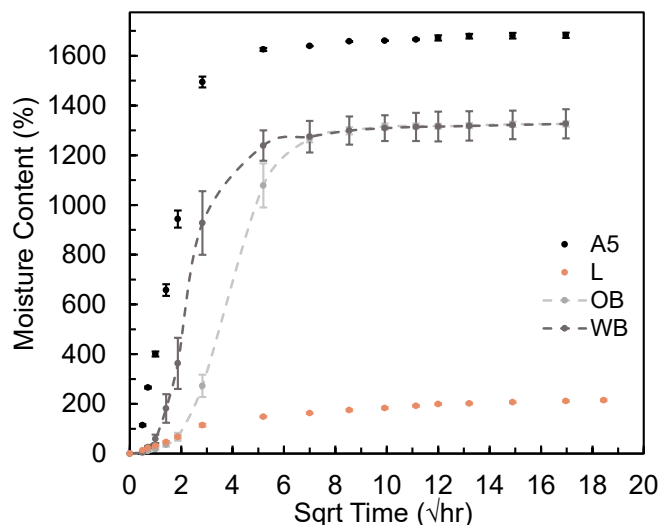


Figure 3.6: Characteristic sorption curves of agar samples physically modified using limestone filler (L) or an oil or water-based polyurethane (OB, WB) at 22 °C. Error bars represent one standard deviation of triplicate measurements.



Figure 3.7: Imperfections in polyurethane coating observed after 6 hours of immersion. Oil-based and water-based coating shown on left and right, respectively.

The final equilibrated moisture content of oil-based and water-based coated samples were 1,326% and 1,327%, respectively. The difference in final moisture content between A5 and polyurethane coated samples is likely due to the mass of the polyurethane coatings themselves, which absorb trivial amounts of moisture. When the moisture content is corrected by excluding the mass of the polyurethane coating from calculations, the final equilibrated moisture content of oil-based and water-based coated samples becomes 1,680% and 1,718%, respectively. The inclusion of 15% w/w limestone filler reduced the moisture sorption of 5% w/w agar from 1,682% to 225%. These findings align with literature on hydrogel-based composites - rigid fillers can increase composite density and alter the swelling capacity of hydrogels (“Chapter 3. Natural Polysaccharide-Based Hydrogels and Nanomaterials” n.d.; Utech and Boccaccini 2016).

Figure 3.8 presents the moisture sorption results for 5% w/w agar samples chemically modified by cross-linking with maleic anhydride (MA). The addition of 1%, 5%, 10%, and 30%

MA decreased the moisture sorption of unmodified agar from 1,682% to 1,634%, 1,459%, 1,087%, and 663%, respectively. This reduction in moisture sorption is due to the reduced number of free -OH groups on the structural chain of agar that are available for hydrogen bonding with water. Additionally, crosslinking with maleic anhydride produces a denser, more fibrillar intermolecular network and increases the rigidity of agar, which can aid in inhibiting swelling (Ye et al. 2022).

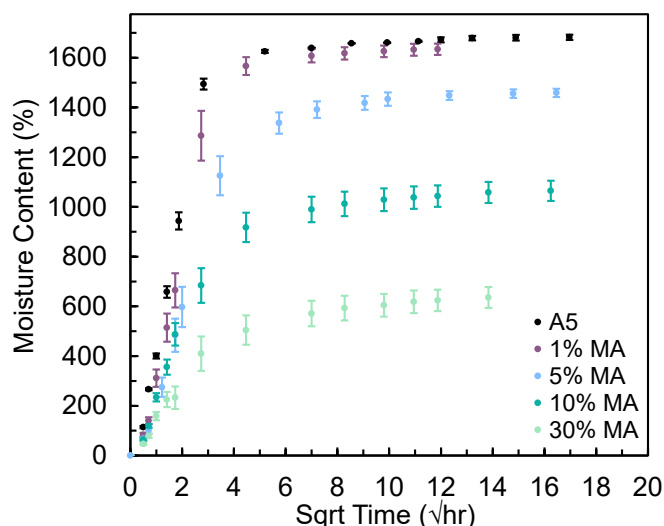


Figure 3.8: Characteristic sorption curves of agar samples chemically modified by crosslinking with 1, 5, 10, and 30% maleic anhydride at 22 °C. Error bars represent one standard deviation of triplicate measurements.

Although increasing the concentration of MA reduced the moisture sorption of unmodified agar, a visible reduction in the effectiveness was observed for higher concentrations of MA (*i.e.*, 10% and 30%). This relationship is illustrated in Figure 3.9 by plotting reduction in moisture content (*i.e.*, relative to sample A5, as a %) versus the MA concentration (*i.e.*, as a %). An increase in MA concentration from 5 to 10% produced an almost identical reduction in moisture sorption as compared to increasing the MA from 10 to 30% (*i.e.*, 22 and 25% difference, respectively). This data illustrates a diminishing moisture sorption benefit in regard to MA concentration and indicates that there may be a saturation point for MA inclusion. It is hypothesized that a portion of the dose in higher MA concentrations is not chemically reacting with agar. In this case, reductions in moisture sorption may be due to unreacted MA acting as a pseudo filler material, similar to physical modification with limestone filler.

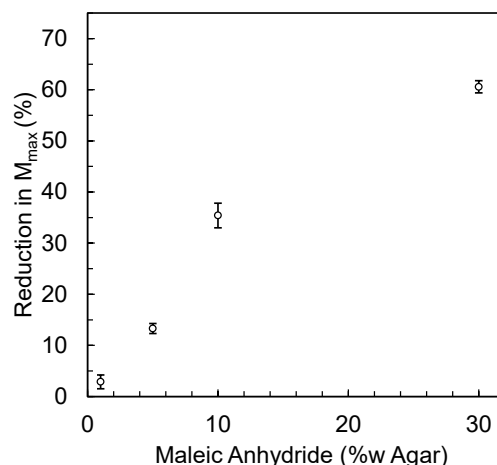


Figure 3.9: Impact of maleic anhydride dosage on the reduction in moisture sorption as compared to an unmodified agar control sample. Error bars represent one standard deviation of triplicate measurements.

Figure 3.10 illustrates the mass loss of reconditioned MA modified samples (*i.e.*, acclimated to constant mass after immersion equilibration, as a %) versus the MA concentration. 1% and 5% MA samples lost 8.4% and 13.2% of mass, respectively, when reconditioned, which is comparable to the mass loss illustrated by sample A5 (*i.e.*, 10.4%, Figure 3.11A). Conversely, reconditioned 10% and 30% MA samples lost 23.9% and 40.5% of sample mass. It is hypothesized that this increase in mass loss is due to leaching of unreacted MA and these findings align with results of moisture sorption testing that indicate a diminishing moisture sorption reduction with higher dosages of MA.

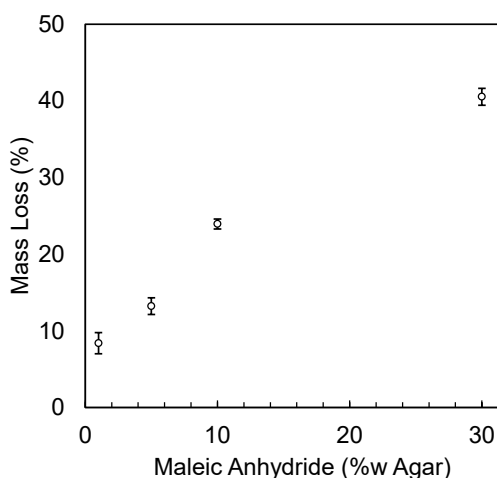


Figure 3.10: Impact of maleic anhydride dosage on the mass loss of samples after reconditioning. Error bars represent one standard deviation of triplicate measurements.

Mass loss for unmodified and physically modified samples are shown in Figure 3.11. Unmodified agar (*i.e.*, panel A) lost 9.2 to 10.4% of conditioned samples mass when reconditioned. While results for physically modified samples (*i.e.*, panel B) indicate that OB samples experienced similar mass loss as compared to A5 (*i.e.*, 8.7% vs 10.4%), WB samples illustrated more mass loss after reconditioning (*i.e.*, mass loss = 17.2%). During immersion testing, WB coatings disintegrated into fragments in the water bath, while OB coatings retained shape after coating failure. The increase in mass loss of WB samples is likely due to the fragmentation of coating material that led to less recovered polyurethane mass after immersion testing.

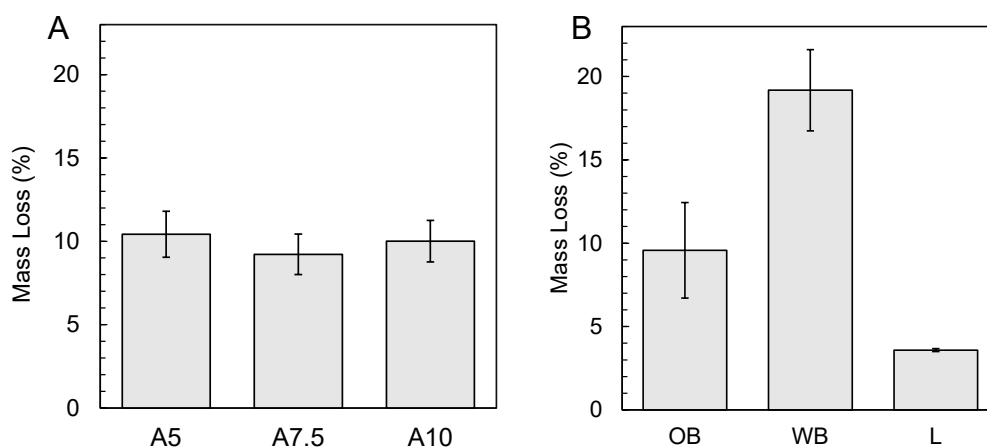


Figure 3.11: Mass loss after reconditioning for (A) unmodified agar and (B) physically modified agar. Error bars represent one standard deviation of triplicate measurements.

The use of limestone filler in samples reduced the mass loss of samples from 10.4% to 3.7% (*i.e.*, A5 compared to L in Figure 3.11A). These results align with findings in Chapter 2 and indicate that agar sample formulations with limestone may have a stronger gel network than formulations without limestone (Labropoulos et al. 2001). Agar samples physically modified with limestone may be able to withstand stresses due to drying shrinkage more successfully than unmodified samples.

Classification and Modeling of Diffusion Behavior

A graphical representation of Equation 3.4 is shown in Figure 3.12 and the slope, intercept, and R^2 of the linear regression for each sample are shown in Table 3.2. Results are shown for all samples with the exception of samples physically modified with a polyurethane coating that did not illustrate typical Fickian or relaxation-controlled behavior. The linear regression analysis

yielded a high R^2 value (*i.e.*, $R^2 > 0.95$) for all samples indicating adequate fit of Equation 3.4 to the experimentally obtained moisture sorption data. Notably, the high R^2 values may be due to the relatively small number of data points (*i.e.*, three to four data points total) available to characterize the initial linear portion of the moisture sorption curves.

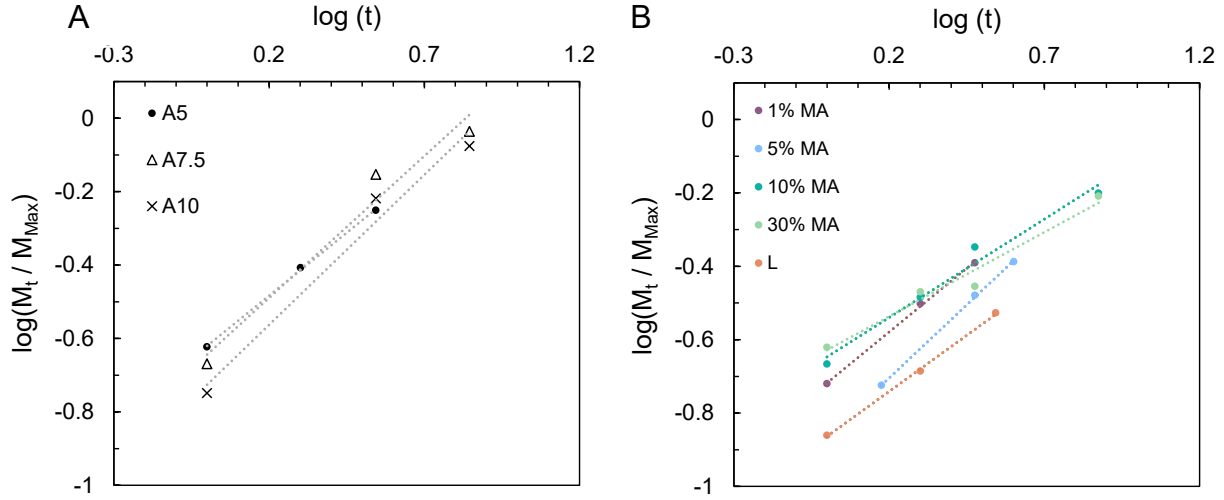


Figure 3.12: Diffusion case analysis according to Equation 3.4 for (A) unmodified and (B) modified agar samples.

Table 3.2: Linear regression constants obtained from diffusion case analysis according to Equation 3.4.

Sample	Slope	Intercept	R^2
A5	0.7710	-0.6412	0.980
A7.5	0.7728	-0.6442	0.966
A10	0.8172	-0.7261	0.976
1% MA	0.8646	-0.7736	0.991
5% MA	0.8113	-0.8745	0.996
10% MA	0.8119	-0.7174	0.993
30% MA	0.6809	-0.7076	0.959
L	0.6038	-0.8502	0.995

The diffusion classification of each sample can be determined from the kinetic rate constant (*i.e.*, n), which is represented by the slope for each linear regression. In this analysis, n varied from 0.6038 (*i.e.*, 50% MA) to 0.8646 (*i.e.*, 1% MA). Values of n indicate that all agar samples exhibited anomalous diffusion behavior (*i.e.*, $0.5 < n < 1$) and that the sorption of moisture is dependent on

polymer relaxation behavior. Kinetic rate constants obtained in this work are in line with literature on biopolymer diffusion- Previous work with Xanthan gum and hydroxypropylmethyl cellulose illustrated values of n ranging from 0.67 to 0.78 (Andreopoulos and Tarantili 2002). Work with gelatin illustrated kinetic rate constants ranging from 0.56 to 0.70 (Hess and Srubar 2016).

Given that values of n suggest anomalous diffusion, further analysis was conducted using the Berens-Hopfenberg differential equation (*i.e.*, Equation 3.5 and 3.6) in order to classify polymer relaxation behavior and more accurately model diffusion behavior for $\frac{M_t}{M_{Max}} > 0.6$. A graphical representation of this analysis is shown in Figure 3.13 and Table 3.3 illustrates the constants and R^2 of the linear regression.

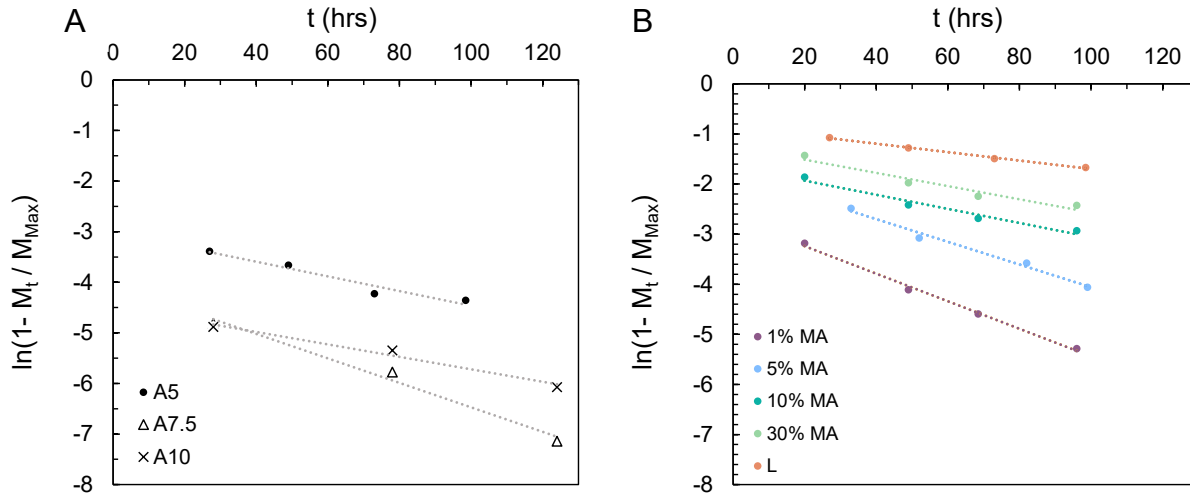


Figure 3.13: Diffusion case analysis according to Equation 3-6 for (A) unmodified and (B) modified agar samples.

Table 3.3: Linear regression constants obtained from diffusion case analysis according to equation 3-6.

Sample	Slope	Intercept	R^2
A5	-0.015	-3.0117	0.944
A7.5	-0.024	-4.0562	0.985
A10	-0.012	-4.4873	0.977
1% MA	-0.028	-2.6873	0.995
5% MA	-0.023	-1.7934	0.986
L	-0.008	-0.8592	0.996

Values obtained for all kinetic constants (*i.e.*, n , k , k_2 , and A) are provided in Table 3.4. Unmodified agar samples illustrated a shift towards more relaxation-controlled diffusion behavior with an increase in agar content (*i.e.*, $n = 0.77 \rightarrow n = 0.82$). These experimental observations align moisture sorption observations- An increase in agar content results in an increase in polymer network density, which can require further relaxation of the polymer network and hinder polymer mobility in the presence of water (Ghebremedhin et al. 2021; Pourjavadi et al. 2009; Rani et al. 2018).

Table 3.4: Diffusion coefficients and kinetic constants used to classify and model diffusion behavior.

Sample	M_{Max} (%)	n	k	Diffusion Classification	k_2	A
A5	1,682	0.7710	0.2397	Anomalous	0.0145	0.0492
A7.5	1,262	0.7728	0.2269	Anomalous	0.0242	0.0173
A10	868	0.8172	0.1879	Anomalous	0.0123	0.0113
1% MA	1,634	0.8646	0.1908	Anomalous	0.0275	0.0681
5% MA	1,459	0.8113	0.1369	Anomalous	0.0227	0.1664
10% MA	1,087	0.8119	0.2255	Anomalous	0.0141	0.1920
30% MA	663	0.6809	0.2352	Anomalous	0.0132	0.2869
L	220	0.6038	0.1369	Anomalous	0.0084	0.4235

Chemically modified agar samples illustrated an initial shift towards more relaxation-controlled diffusion behavior (*i.e.*, A5 compared to 1% MA, $n = 0.77 \rightarrow n = 0.86$). A subsequent increase in the maleic anhydride (MA) concentration of samples shifted diffusion behavior from polymer-relaxation controlled to a more Fickian behavior (*i.e.*, 1% MA compared to 30% MA, $n = 0.86 \rightarrow n = 0.68$). An increase in MA content led to greater dependence on moisture diffusion rather than polymer relation, which may be due to lower reactivity of the agar structure (*i.e.*, reduced number of available hydroxyl groups) and an increase in network density.

Samples physically modified with limestone filler exhibited behavior closest to Fickian diffusion (*i.e.*, $n = 0.60$) and these samples were less reliant on polymer relaxation to transport moisture. Notably, diffusion behavior of agar modified with 30% MA led to diffusion behavior similar to sample L (*i.e.*, $n = 0.68$ and $n = 0.6$, respectively). These results align with moisture sorption and mass loss findings that indicate a diminishing moisture sorption reduction and increase in mass loss with higher dosages of MA and a hypothesized filler effect.

Values obtained in diffusion analysis and shown in Table 3.4 were used to create models to predict water transport behavior. This was accomplished in a piecewise manner, by fitting Equation 3.4 to $\frac{M_t}{M_{Max}} < 0.6$ and Equation 3.6 to $\frac{M_t}{M_{Max}} \geq 0.6$ (Ganji et al. 2010; Hess and Srubar 2016). Diffusion models for unmodified, physically modified, and chemically modified agar samples are overlaid onto experimental moisture sorption curves to illustrate fit and shown in Figure 3.14, Figure 3.15, and Figure 3.16, respectively.

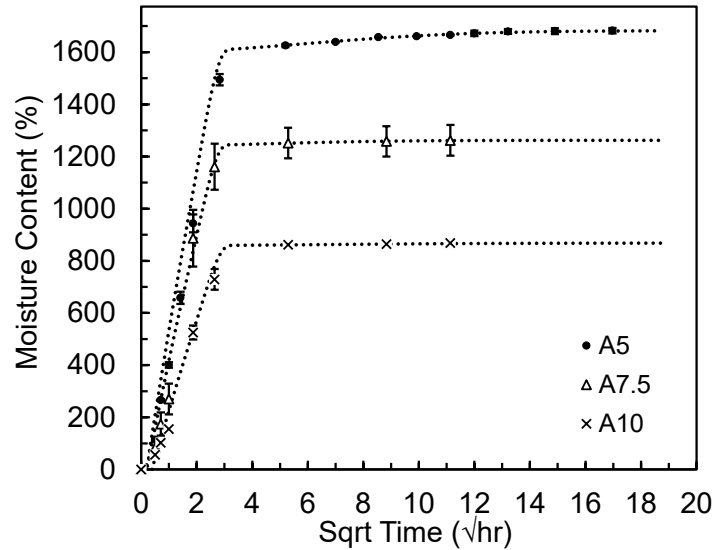


Figure 3.14: Characteristic sorption curves of 5, 7.5, and 10% w/w agar at 22 °C overlaid with diffusion model outputs, shown as a dashed line.

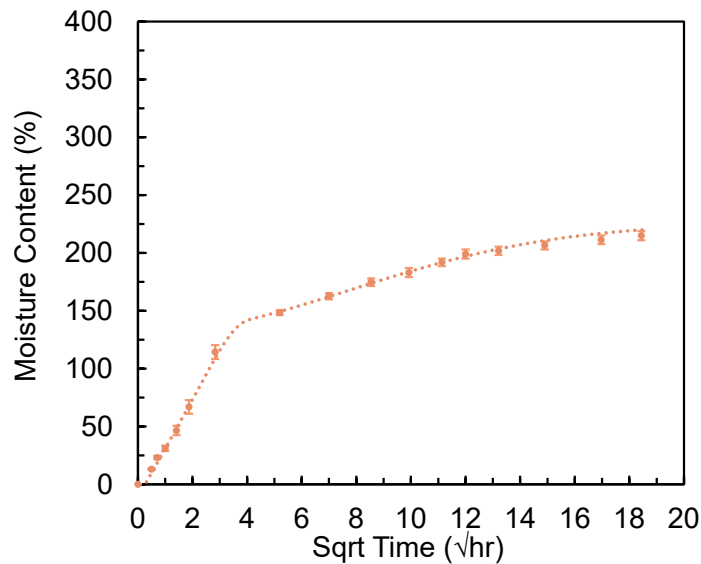


Figure 3.15: Characteristic sorption curves of physically modified (*i.e.*, dosed with an inert limestone filler) at 22 °C overlaid with diffusion model outputs, shown as a dashed line.

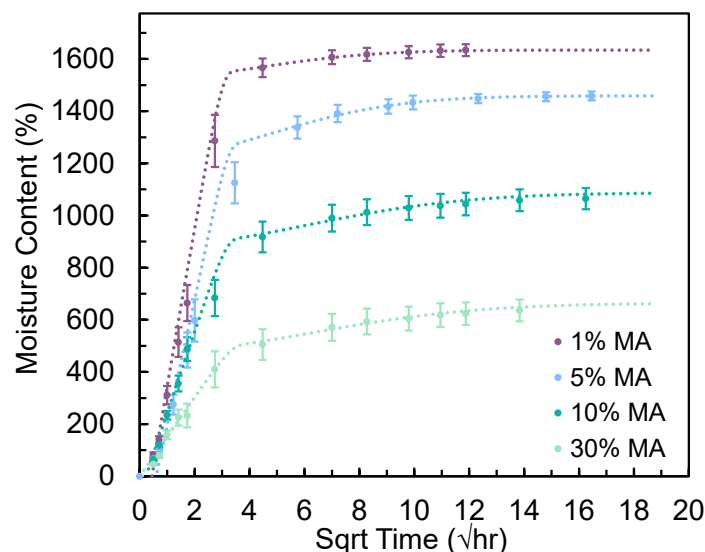


Figure 3.16: Characteristic sorption curves of chemically modified (*i.e.*, crosslinking with 1, 5, 10, and 30% maleic anhydride) at 22 °C overlaid with diffusion model outputs, shown as a dashed line.

3.5 Conclusions

In moisture sorption testing, agar-based materials were found to absorb over 1,600% moisture relative to their dehydrated mass. In this work, the hydrophilicity of agar-based binders was modified through the use of limestone filler, polyurethane coatings (*i.e.*, oil-based and water-based), and chemical cross-linking with maleic anhydride. FTIR was used to verify modification methods- The OH bands of chemically modified samples were found to decrease in intensity, verifying chemical modification methods.

Of the following methods, the addition of 15% w/w limestone filler was found to be the most effective and reduced moisture sorption from 1,682% to 215%. In addition to increased efficacy in moisture sorption reduction, the use of 15% w/w limestone filler is accompanied by less significant deleterious environmental or function impacts on agar-based binder composites. The use of polyurethane and maleic anhydride in agar-based materials is accompanied by trade-offs in the environmental impact and material properties, respectively. Although polyurethane materials can be derived from natural sources like lignin and vegetable oils including castor, soybean, and sunflower oil (Reghunadhan and Thomas 2017), polyurethane materials are more commonly produced using petroleum-based raw materials (Enderus and Tahir 2017). Similarly, although maleic anhydride can decrease agar hydrophilicity, it can come at the cost of reducing other functional properties like gel strength (Ye et al. 2022a). Notably, the addition of 15% w/w

limestone filler is accompanied by less significant deleterious environmental or function impacts on agar-based binder composites- Results obtained in Chapter 2 actually indicate improved thermal stability, penetration, and dimensional stability with the use of limestone filler.

This work provides models that can aid in utilizing agar in civil engineering applications and give practitioners an idea of expected moisture behavior for agar-based materials. Fickian modeling can provide an indication of what is happening molecularly within samples and how modification methods are impacting sample diffusion. Although agar-based binders are not moisture resistant, these results indicate that steps can be taken to modify agar-based materials and improve hydrophilic behavior.

4. Mechanical and Microstructural Characterization of Agar-Based Composites for Pavement Applications

4.1 Introduction

Increased interests in industrial sustainability combined with a decrease in availability of high-quality geotechnical materials has led to an increased interest in biopolymers. Biopolymers like xanthan gum (Jiang et al. 2022; Soldo et al. 2020), guar gum (Muguda et al. 2017), sodium alginate (Arab et al. 2019), gelatin (Vishweshwaran and Sujatha 2023), and agar have been investigated as stabilizers for sands, clays, silts, mine tailings (Chen et al. 2015), and municipal solid waste fines (Verma et al. 2023). Literature notes the ability of biopolymers to improve shear and compressive strength, compressibility, and aid in dust control. A comprehensive review of geotechnical stabilization using biopolymers can be found in Fatehi et al. (2021) and Chang et al. (2020).

Notably, the majority of this literature has focused on general soil stabilization rather than pavement specific applications, with a few exceptions. Arab et al. (2019) evaluated clayey soils stabilized with 2 and 4% sodium alginate for use as a stabilized layer in pavement applications. The compressive strength of treated soil was 1.6 times that of the untreated control, but authors note a decrease in the peak strain of sodium alginate treated samples. Hamza (2023a: b) investigated stabilizing weak, clay-based subgrade materials with 1 to 5% xanthan gum and noted an increase in both the compressive strength and energy absorption capacity. Cabalar et al. (2023) evaluated standard crushed rock base course combined with 1, 3, and 5% xanthan gum for use as a pavement subgrade. The use of xanthan gum raised the compressive strength of control aggregate and increased the energy absorption capacity.

Simultaneously, largescale cultivation of algae represents an incredible opportunity for the production of multiple high value products as a side stream for biofuel. The growth period of algae contributes to the large-scale adsorption of CO₂ without creating competition with agriculture or land use (Laurens et al. 2020). Agar, a biopolymer extracted from red seaweed belonging to the Rhodophyceae class of algae, may be well suited as an alternative material for civil engineering applications.

Agar has been investigated previously as a stabilizing material to improve the engineering properties of geotechnical materials. Smitha et al. (2021) investigated the behavior of silty sand treated with 0.5, 1, and 2% agar biopolymer at multiple curing time periods and noted a significant

increase in cohesion with an increase in biopolymer content and curing time. Chang et al. (2015a) evaluated the use of agar and gellan gum biopolymer with both clayey and sandy soil and noted significant improvements in composite compressive strength as compared to unmodified soil samples. The authors note that this is due to the ability of the biopolymers to coat aggregate surfaces, fill pore spaces, and improve particle to particle contact. Khatami and O’Kelly (2013) evaluated sand combined with 1, 2, and 4% agar by weight and noted an increase in compressive strength from 150 kPa to approximately 500 kPa. Work completed by Fatehi et al. (2021) evaluated agar biopolymer combined with both sand and soil and found that that biopolymer use can improve the compressive strength by 115%. Verma et al. (2023) evaluated the use of xanthan gum and agar to stabilize municipal solid waste fines and noted that agar produced a denser profile than xanthan gum and produced better mechanical performance. Kantesaria et al. (2015) investigated the use of 2% agar in expansive soil. In SEM testing, agar biopolymers coated soil particles and connected aggregate that was not initially in contact.

The water affinity of biopolymers has been found to aid the mechanical properties of soils with liquefaction potential by increasing moisture retention and surface strength (Smitha 2021). Work by Kantessaria et al. (2015) found that agar-stabilization reduced swelling potential and pressure in expansive soils. Work by Smitha and Sachan (2016) with agar and sand composites noted small reductions in shear strength with composite saturation. Similarly, Fatehi et al. (2021) found that the saturated strength of agar composites was less than that of dehydrated composites but was still higher than that of untreated saturated samples. Further research is needed to capitalize on the promising benefits of agar and understand the longevity and effect of agar-based composites (Chen et al. 2015) in the direct context of traditional pavement materials.

4.2 Research Objectives

The objectives of this research are to evaluate the microstructure and mechanical properties of agar-based composites and to derive preliminary estimations of costs and CO₂ emissions associated with the cultivation and processing of agar materials. This characterization is crucial to understand how agar-based composites compare to traditional engineering materials and other biobased composites and identify specific applications where agar-based materials could constitute a sustainable alternative.

In particular, this work investigates the properties of composites comprised of 5% w/w agar combined with a typical aggregate mixture specified for use in Coloradan pavement materials.

Composite properties were evaluated at two binder ratios (*i.e.*, mass ratios of 0.2 and 0.5) with and without two filler materials (*i.e.*, limestone powder, Ottawa sand). The microstructure (*i.e.*, porosity, pore network characteristics) of agar-based composites were evaluated by non-destructive X-ray tomography testing. Mechanical properties were evaluated through unconfined compressive strength testing and data was further processed to obtain sample stress-strain curves and calculate the modulus of elasticity and resilience. Results are compared to traditional engineering materials and other biobased composites in literature. Finally, this work provides context on the relative cost and CO₂ emissions associated with the cultivation, transport, and processing of agar materials.

4.3 Materials and Methods

Materials

Agar powder, reagent-grade limestone (calcium carbonate, CaCO₃) with a particle size < 250 µm, and Ottawa sand µm were supplied by Sigma Aldrich (St. Louis, MO, USA), Research Products International (Mt Prospect, IL, USA), and Gilson (Middleton, WI, USA) respectively. Ottawa sand is a rounded silica sand, mined and collected from Ottawa, Illinois area (Chocron et al. 2021). Ottawa sand was chosen for evaluation in agar-based composites because it is homogenous and mineralogically pure and illustrates less material variability as compared to in situ sources. Additionally, Ottawa sand is widely used in material laboratories (*i.e.*, as a standardized material for compressive strength testing of mortar by ASTM C109 (C01 Committee n.d.)) making it familiar and available to the construction industry (Erdoğan et al. 2017).

The aggregate used in agar-based composites mixtures was supplied by the Colorado Department of Transportation (CDOT). This aggregate was chosen for this analysis because it represents a commonly used and accepted aggregate for civil engineering applications. This aggregate was collected as a “belt cut” from a Coloradan asphalt manufacturing plant. In this sampling method, the aggregate was collected randomly from the conveyer belt of an asphalt plant prior to being mixed with any asphalt binder (D04 Committee n.d.).

The mechanical response of aggregate composites is largely a function of composite density, which is highly related to the aggregate gradation (Cunningham et al. 2013). To this end, the particle size distribution curve of aggregate used in agar-based composites was characterized by the Colorado Department of Transportation (CDOT) materials laboratory using AASHTO T 27 testing procedure (“AASHTO T 27: Standard Method of Test for Sieve Analysis of Fine and

Coarse Aggregates” n.d.). The percent mass retained on each standard sieve was recorded and converted to the percent passing each sieve. These data are plotted on a logarithmic scale according to the sieve size in order to produce a particle size distribution curve. The particle size distribution curve was used to characterize aggregate gradation and packing properties.

The particle size distribution curve of graded aggregate used to form agar-based composites is shown in Figure 4.1. The particle size distribution curve illustrates a wide range of aggregate sizes and an “s” shape that is typical for a dense-graded aggregate mixture (Fang et al. 2019). In a dense-graded aggregate structure, loading is transferred through particle to particle contact of coarse and fine aggregate (Powrie 2014). As aggregates have very little cohesion, the shear strength of a gradation is primarily provided by the internal friction of the aggregate (Asphalt institute 2001). Dense grading creates a highly packed aggregate structure that is advantageous for improving the mass stability, shear strength, and elastic stiffness of composites in engineering applications (Cunningham et al. 2013).

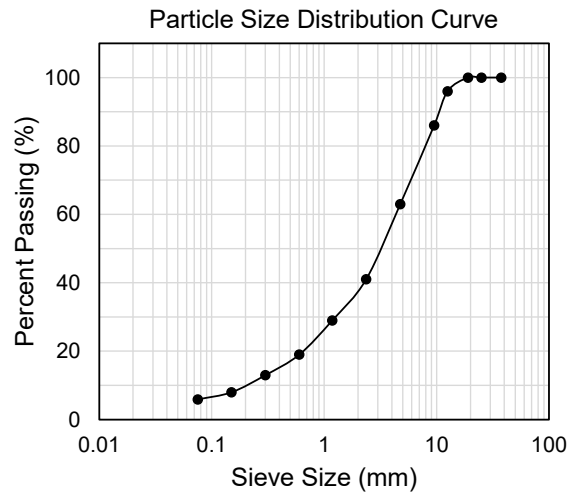


Figure 4.1: Particle size distribution curve for aggregate obtained from the Colorado Department of Transportation (CDOT).

Methods

Sample Preparation

Composite formulations, designed by mass ratios, are outlined in Table 4.1. The mass ratio of graded aggregate and reinforcing materials (i.e., cumulative mass of aggregate, limestone, and Ottawa sand) was kept constant at a ratio of 1.0 for all composites in order to maintain overall sample ratios and allow for better comparability between sample types. Sample subsets reinforced

with limestone or sand fillers were prepared at constant mass ratios of 0.05 and 0.2, respectively and the mass ratio of graded aggregate varied from 0.75 to 1.0. Composites were prepared at two mass ratios of 5% w/w agar binder, namely 0.2 and 0.5. This resulted in a total of eight composite sample types. The sample naming convention is as follows: C for control samples (i.e., formulations with only aggregate), L for limestone-containing samples, S for Ottawa sand-containing samples, and LS for limestone- and Ottawa sand-containing samples. The letter designation is followed by the mass ratio of agar binder (i.e., 0.2 or 0.5).

Table 4.1: Mass ratios of constituent mix components for agar-based composites.

Subset	Sample Name	5% w/w Agar Binder	Aggregate	Limestone	Sand
Control	C-0.2	0.2	1	-	-
	C-0.5	0.5	1	-	-
Limestone Filler	L-0.2	0.2	0.95	0.05	-
	L-0.5	0.5	0.95	0.05	-
Sand Filler	S-0.2	0.2	0.80	-	0.2
	S-0.5	0.5	0.80	-	0.2
Limestone and Sand Filler	LS-0.2	0.2	0.75	0.05	0.2
	LS-0.5	0.5	0.75	0.05	0.2

Agar was prepared at a 5% w/w concentration relative to deionized water. 600 ml of deionized water was added to a 1 liter media bottle and 30 g of agar powder was dissolved at ambient laboratory conditions (22 ± 2 °C) under continuous agitation using a magnetic stir bar. The media bottles were then placed in an SK101C Yamato autoclave (Santa Clara, CA, USA) and heated to 100 °C for 20 min.

After autoclaving, the media bottles were removed and the appropriate amount of gelled agar was weighed out to reflect the mass ratios (i.e., 0.2 or 0.5) outlined in Composites prepared with limestone or Ottawa sand filler were mixed by hand into the agar for approximately 1 minute until filler materials were well dispersed. The appropriate amount of aggregate (i.e., 0.75, 0.80, 0.95, or 1.0 mass ratio) was weighed and mixed with agar-based materials in Hobart mechanical mixer for approximately 1-min. Sample mixtures were compacted into 2-in cube molds in three

layers and tamped by hand after the addition of each layer. Samples were allowed to gel for approximately 30-min and then removed from molds.

Composites were acclimated at ambient laboratory conditions (i.e., 22 ± 2 °C) until reaching constant mass, defined as less than 1% change in composite mass in a 24-hr period. Since the mechanical properties of agar-based composites have been shown to improve with dehydration to a dry state (Huang et al. 2021a), the composites were acclimated to constant mass to replicate the best conditions for loadbearing or structural applications and to ensure all samples were at a consistent, reproducible moisture state. Images of C-0.2 and C-0.5 samples directly after de-molding and after acclimation to constant mass are shown in Figure 4.2.

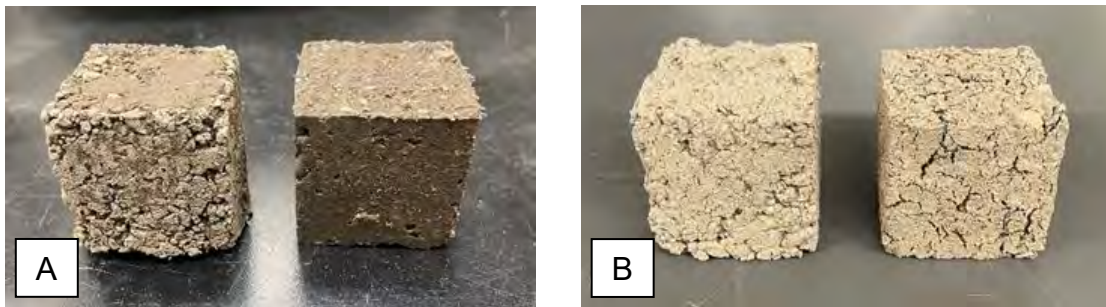


Figure 4.2: Prepared agar-based composites (A) after de-molding and (B) after acclimation to constant mass.

X-Ray Tomography

The porosity of agar-based materials was not evaluated using typical water displacement methods (i.e., ASTM D7063, ASTM C642) due to the hydrophilicity of agar-based materials that could alter the sample properties. X-ray tomography was chosen as a nondestructive alternative to evaluate the aggregate packing structure and sample porosity. 3-D visualization of the internal structure of agar-based composites was obtained through X-ray tomography testing using a ZEISS Xradio 520 Versa system. The source voltage was set to 140 kV and the power to 10 W. An optical magnification of 0.4X and an HE3 filter were used to produce images. Agar-based composites prepared for X-ray tomography analysis are shown in Figure 4.3.

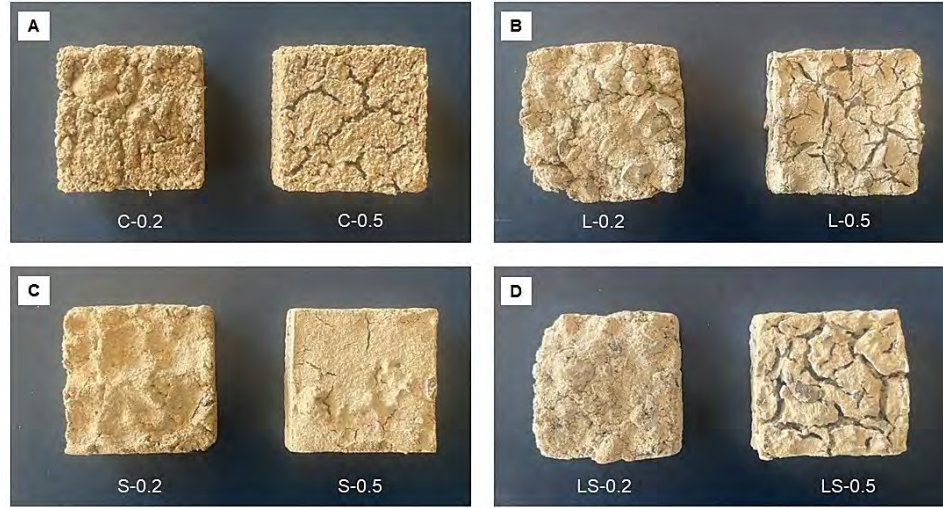


Figure 4.3: Representative images of samples prepared for X-ray tomography testing. Panels show A) control samples, B) limestone reinforced samples, C) sand reinforced samples, and D) limestone and sand reinforced samples.

X-ray tomography is conducted by projecting a cone beam of X-rays onto a sample and capturing projections on a detector. The sample is mounted between the X-ray source and detector and is rotated throughout testing to create renderings of 2D “slices” of the sample (du Plessis and Boshoff 2019; Salvo et al. 2010). An image of the testing setup is shown in Figure 4.4. Upon completion of X-ray tomography testing, 2D slices are “stacked” and processed to create a 3D reconstruction of the sample. The X-ray density captured by the detector is related to the physical density of the sample, as denser objects appear brighter in readings. Due to this premise, X-ray tomography is frequently applied to identify pore spaces in samples and to quantitatively analyze the spatial distribution and size of pores [8]. This method has been applied to cement paste (Frazier et al. 2020), concrete (Koenig 2020), and asphalt (du Plessis and Boshoff 2019).

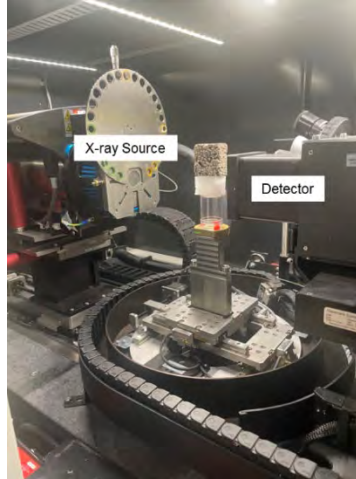


Figure 4.4: Experimental setup for X-ray tomography testing.

X-ray tomography analysis of pores requires processing to segment images at delineation thresholds [8]. Dragonfly 2022.2 software (Object Research Systems) was used to create 3D reconstructions and segment images of agar-based composites to produce volumetric data. The volume of air voids (i.e., pores) in samples was determined by splitting the image pixel intensity at the Otsu threshold (Xu et al. 2011) two times. The first split segmented data into dense particles (i.e., aggregate) and less dense components (i.e., binder, filler, and voids). The second split, conducted on the foreground (i.e., other or binder, filler, and voids) segmented data into binder and filler versus pores. The segmentation process is illustrated in Figure 4.5.

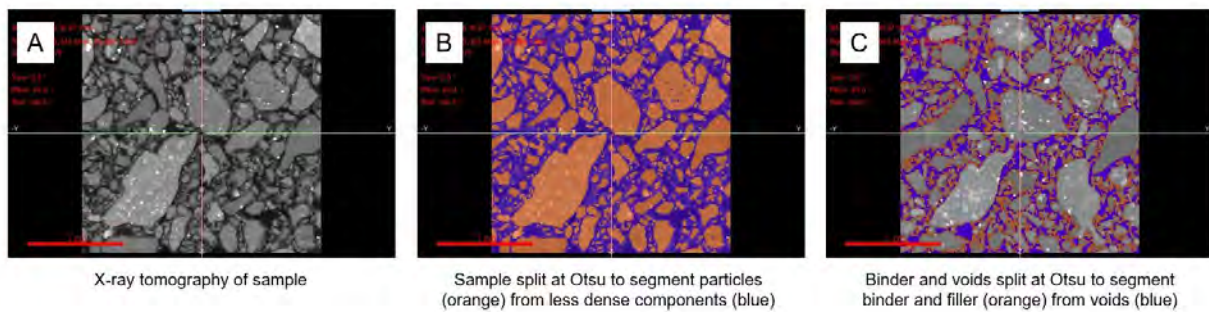


Figure 4.5: Segmentation processing implemented to analyze the porosity of agar-based composites. Samples were split at the Otsu threshold two times to segment composite components. Scale bar = 1 cm.

The volume percentage of pores in samples was determined by comparing the volume of segmented air voids against the total sample volume. Segmented sample data were further processed to create volume thickness maps of the pore space in order to analyze the pore sizes and distribution. Volume thickness mapping is an image processing technique based on a sphere fitting

method that creates a “map” by identifying the diameter of the largest sphere that can be bounded within the region of interest and assigning distance values with color values (Object Research Systems 2019). This technique was applied to the region of pores segmented from the internal structure of agar-based composites to provide a colorized map of the internal pore network.

X-ray tomography testing requires the determination of a representative volume element (RVE), namely the minimum volume required for scanning that represents the bulk properties of the sample. In order to understand impacts from sample size scaling, the volumetric porosity was measured from the reconstruction of the entire tested composite (i.e., 50 x 50 mm by 25-mm sample) as well as from a smaller internal cross section sampled from the center of the tested composite (i.e., 25 x 25-mm by 15-mm). Volume thickness maps were created based on reconstructions of the smaller internal cross-section.

Unconfined Compressive Strength

The unconfined compressive strength of agar-based composites was obtained using an Instron Universal Testing Machine and a constant axial extension rate of 0.1 mm/s. Prior to compressive strength testing, the average height (i.e., as an average of all 4 sides) and surface area (i.e., top and bottom) were measured using calipers. The compressive strength was calculated by dividing the maximum compressive load by the average sample test area (A_{Avg}). Stress-strain curves were calculated from compressive strength data using the following equations:

$$Stress (\sigma) = \frac{F}{A_{Avg}} \quad \text{Equation 4.1}$$

$$Strain (\varepsilon) = \frac{\Delta L}{L_0} \quad \text{Equation 4.2}$$

Where F represents the compressive force applied during testing, L_0 represents the average height of the sample prior to testing, and ΔL represents the change in sample length during testing in the linear elastic regime.

Modulus of Elasticity and Resilience

Stress-strain curves derived using Equations 4.1 and 4.2 were used to calculate the modulus of resilience (U_r) and modulus of elasticity, or Young’s modulus, (E) for agar-based composites. The modulus of elasticity is a material parameter of stiffness that quantifies the amount of stress a material can withstand per unit of strain before deforming plastically (Craig 2000). E can be found by calculating the ratio of stress to strain in the linear elastic portion of the stress-strain curve (i.e., the slope of the linear region) and is quantified as:

$$E = \sigma / \epsilon \quad \text{Equation 4.3}$$

The modulus of resilience (U_r) of agar-based composites was evaluated in order to understand elastic characteristics. U_r characterizes the strain-energy per unit volume of a material within the elastic range and quantifies the amount of energy a material can absorb without deforming plastically (*i.e.*, permanently). The term “resilience” is used to indicate the ability of a material to absorb and release the energy in this range (Gere and Goodno 2013). Generally, U_r is defined as the area under the elastic portion of the stress strain curve. For uniaxial stress, this can be quantified according to equation 4.4:

$$U_r = \frac{1}{2} \sigma \epsilon \quad \text{Equation 4.4}$$

The elastic portion of each stress-strain curve was found by fitting a line through two points: The data point representing 40% of the maximum stress and the elastic limit, defined as 80% of the maximum stress. Using data from a linear fit corrects for the initial curved portion of the stress-strain curve that forms due to surface irregularities (Hossain and Kim 2014). An illustration of this method is shown in Figure 4.6 with the modulus of resilience shown in gray.

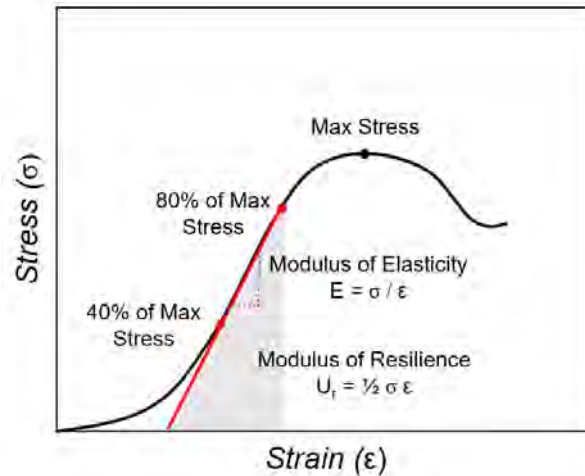


Figure 4.6: Illustration of methodology used to calculate the modulus of elasticity (E) and modulus of resilience (U_r - shown in gray).

Mechanical characterization results were analyzed through Scheirer Ray Hare (SRH) statistical techniques using a 95% confidence interval. SRH is a non-parametric equivalent of two-way ANOVA. This statistical methodology was chosen because the assumptions of parametric testing through ANOVA could not be met (*i.e.*, normality). The two factors considered were i) binder content (*i.e.*, 0.2 or 0.5) and ii) aggregate and reinforcement material composition (*i.e.*, control, limestone reinforced, sand reinforced, or limestone and sand reinforced). Sample data determined

to be statistically significant (*i.e.*, $p \text{ value} \leq 0.05$) were further analyzed in post hoc testing to highlight statistically significant pairwise comparisons. A Dunn's multiple comparison test was applied post hoc to compare pairwise differences.

4.4 Results and Discussion

X-Ray Tomography

X-ray tomography reconstructions of the cross-section sampled from the center of each sample are illustrated in Figure 4.7 to Figure 4.10 for control, limestone reinforced, sand reinforced, and limestone and sand reinforced samples, respectively. Each figure provides an image of the reconstructed sample and a volume thickness map of the segmented interior pore network (*i.e.*, top and bottom of figure, respectively). The volume thickness maps use a color spectrum ranging from a dark navy to red or white with the navy coloring representing the smallest measured distances between pores and a red or white color representing the largest distance between pores, measured in mm.

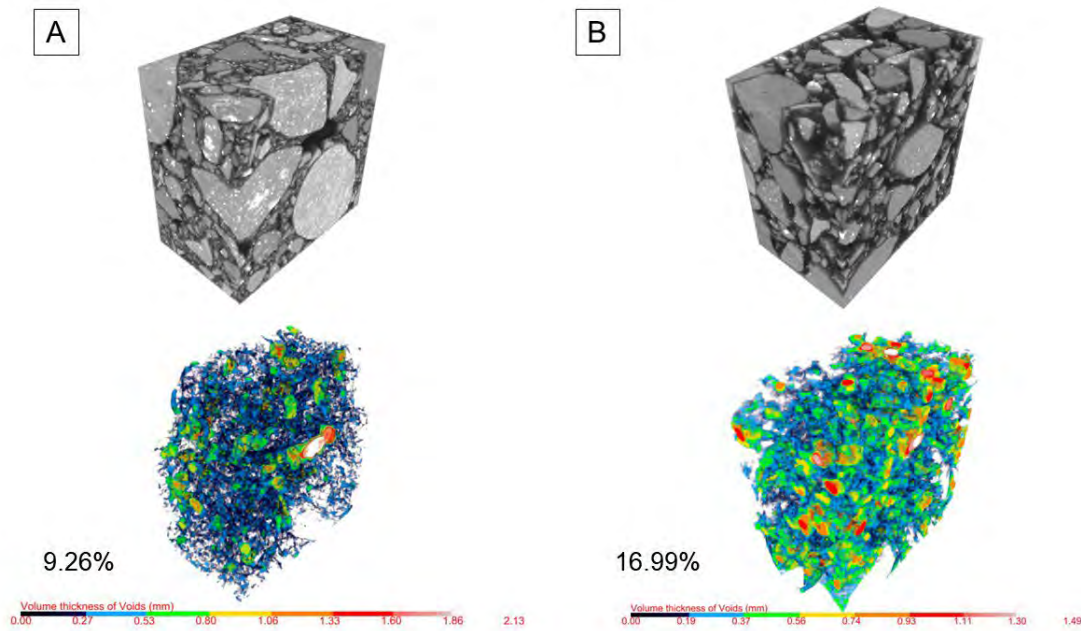


Figure 4.7: X-ray tomography reconstructions and volume thickness map of pore network for *control* composites with (A) 0.2 agar binder content and (B) 0.5 agar binder content. Sample porosity as % volume is shown to the bottom left.

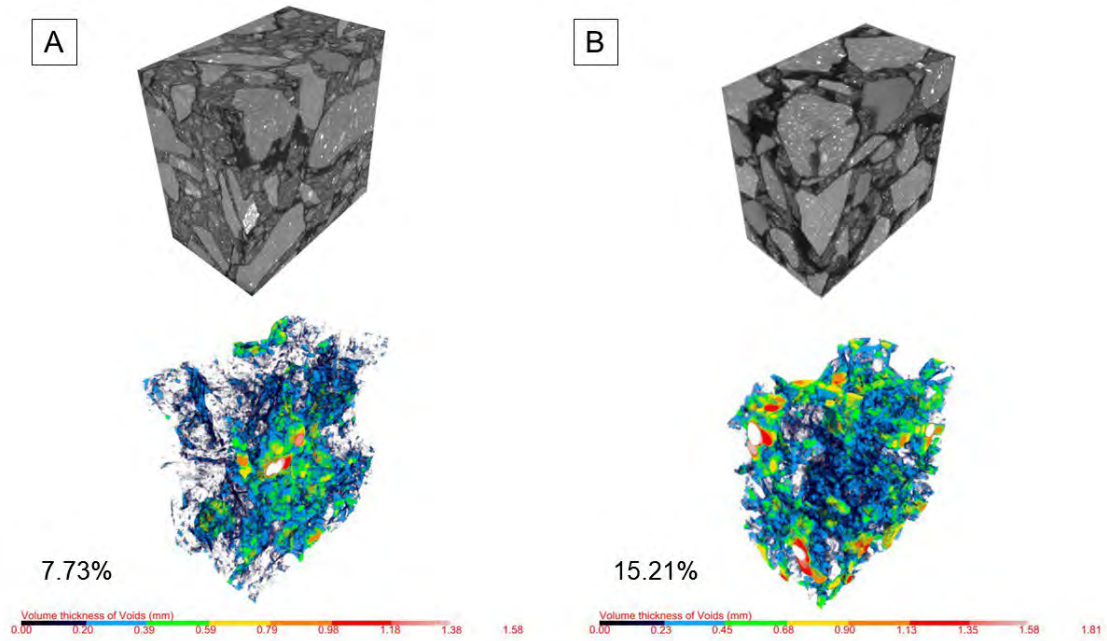


Figure 4.8: X-ray tomography reconstructions and volume thickness map of pore network for *limestone reinforced* composites with (A) 0.2 agar binder content and (B) 0.5 agar binder content. Sample porosity as % volume is shown to the bottom left.

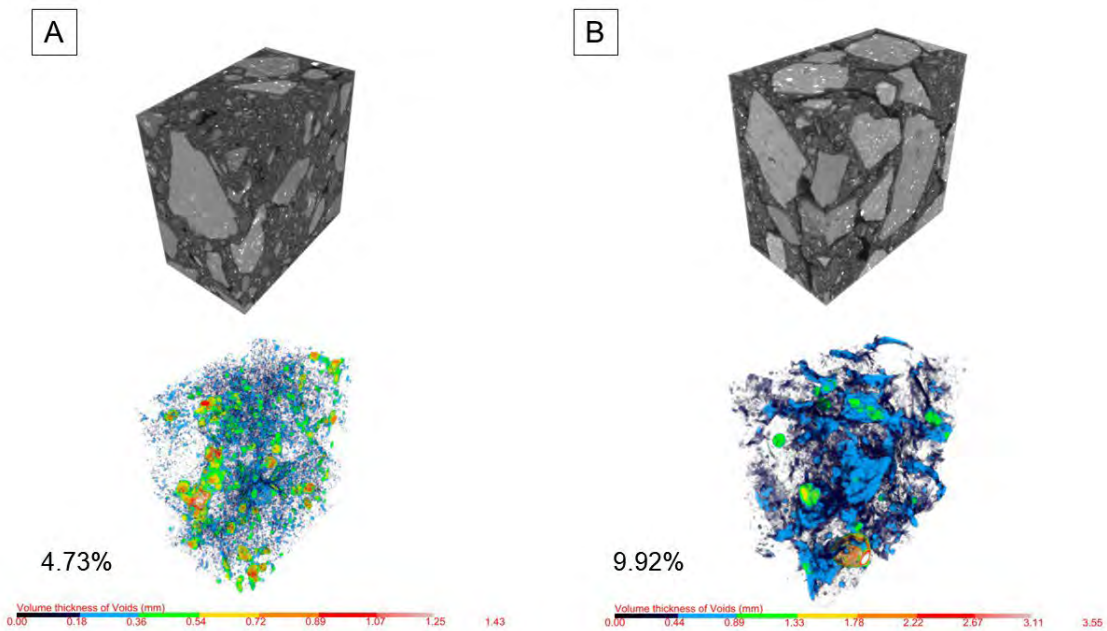


Figure 4.9: X-ray tomography reconstructions and volume thickness map of pore network for *sand reinforced* composites with (A) 0.2 agar binder content and (B) 0.5 agar binder content. Sample porosity as % volume is shown to the bottom left.

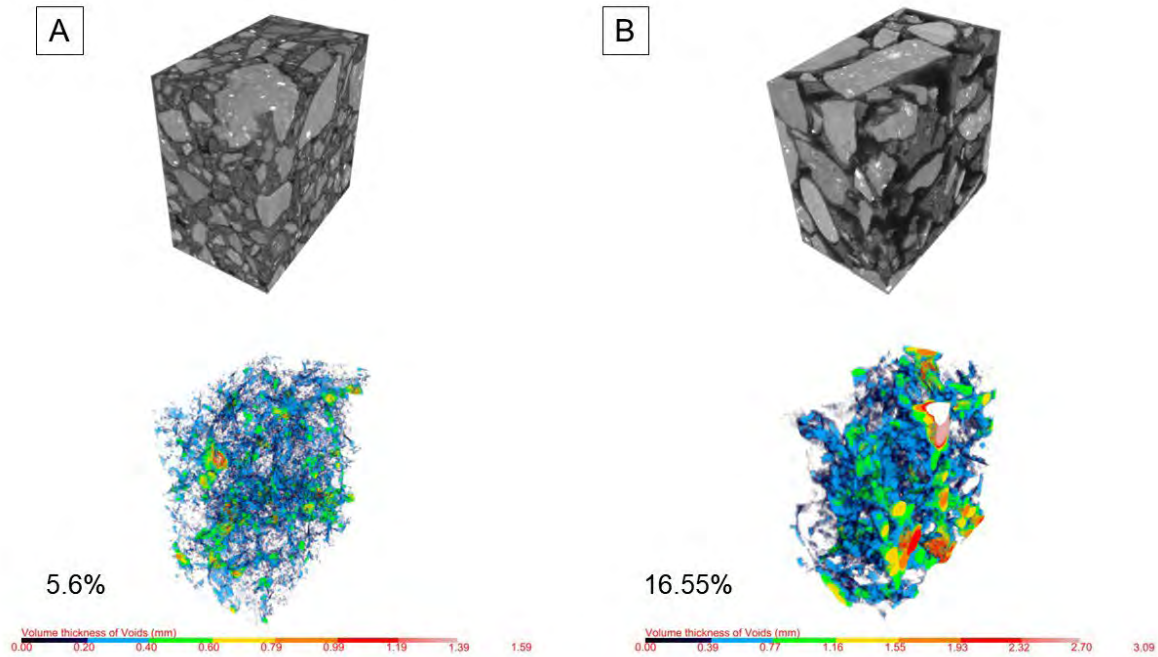


Figure 4.10: X-ray tomography reconstructions and volume thickness map of pore network for *limestone and sand reinforced* composites with (A) 0.2 agar binder content and (B) 0.5 agar binder content. Sample porosity as % volume is shown to the bottom left.

The porosity of agar-based composites ranged from 5.79 to 16.99% for control samples, 6.25 to 15.21% for limestone-containing samples (L), 3.39 to 9.92% for sand-containing samples (S), and 5.60 to 16.55%, for limestone and sand containing (LS) samples. Comparatively, the measured porosity of agar-based composites is generally higher than the porosity of traditional concrete and asphalt (*i.e.*, 5-15% and 2-7%, respectively). However, porosity measurements are within the same range as pervious concrete and porous asphalt (*i.e.*, 15-25% and 10-15%, respectively) (Chen and Yang 2020; Moretti et al. 2019)

Porosity measurements obtained from segmented reconstructions of the entire tested composite (*i.e.*, filled marker) and from a smaller internal cross section sampled from the center of the sample (*i.e.*, unfilled marker) are shown in Figure 4.11. In general, the porosity measured from the smaller segmented volume of the sample was slightly higher than the porosity calculated based on the entire sample volume. The LS-0.2 sample (*i.e.*, limestone and sand reinforced, 0.2 binder content) was the only sample that presented an exception to this trend (*i.e.*, porosity of the entire composite volume was approximately equivalent to the porosity of the segmented composite volume).

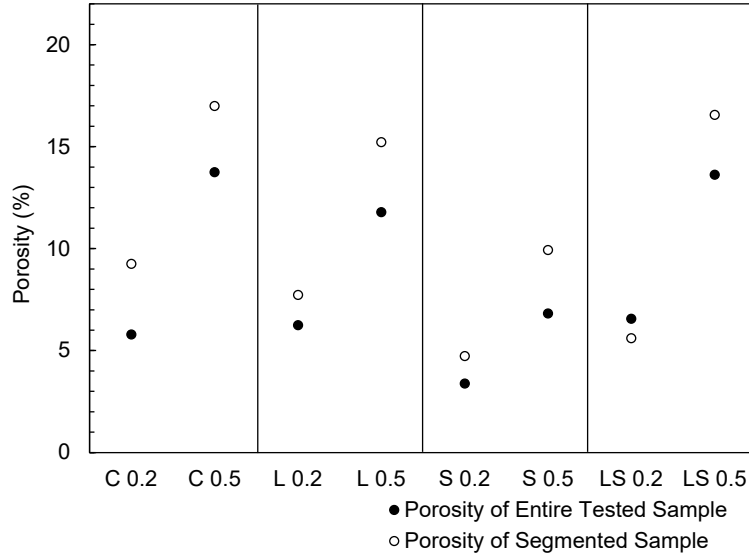


Figure 4.11: Porosity (% volume) of agar-based composites tested in X-ray tomography. Results shown represent the porosity calculated from a reconstruction of the entire test sample (*i.e.*, 50 x 50 by 25-mm) and from a smaller volume segmented from the center of the test sample (*i.e.*, 25 x 25 by 15-mm).

The volume thickness maps shown in Figure 4.7 to Figure 4.10 identify the diameter of the largest sphere that can be bound within the segmented pore space of composite reconstructions. The maximum diameter of pores in samples ranged from 1.4 to 2.1 mm for all agar-based composites, with the exception of S-0.5 and LS-0.5 composites. The maximum diameter of pores for these samples was larger and ranged from 3.1 to 3.6 mm. Detailed diameter data was processed in order to compute the 50th percentile (*i.e.*, median) pore diameter for each sample and results are illustrated in Figure 4.12.

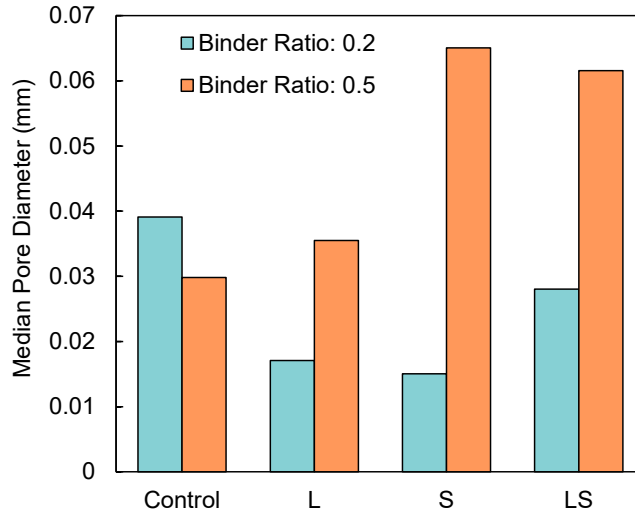
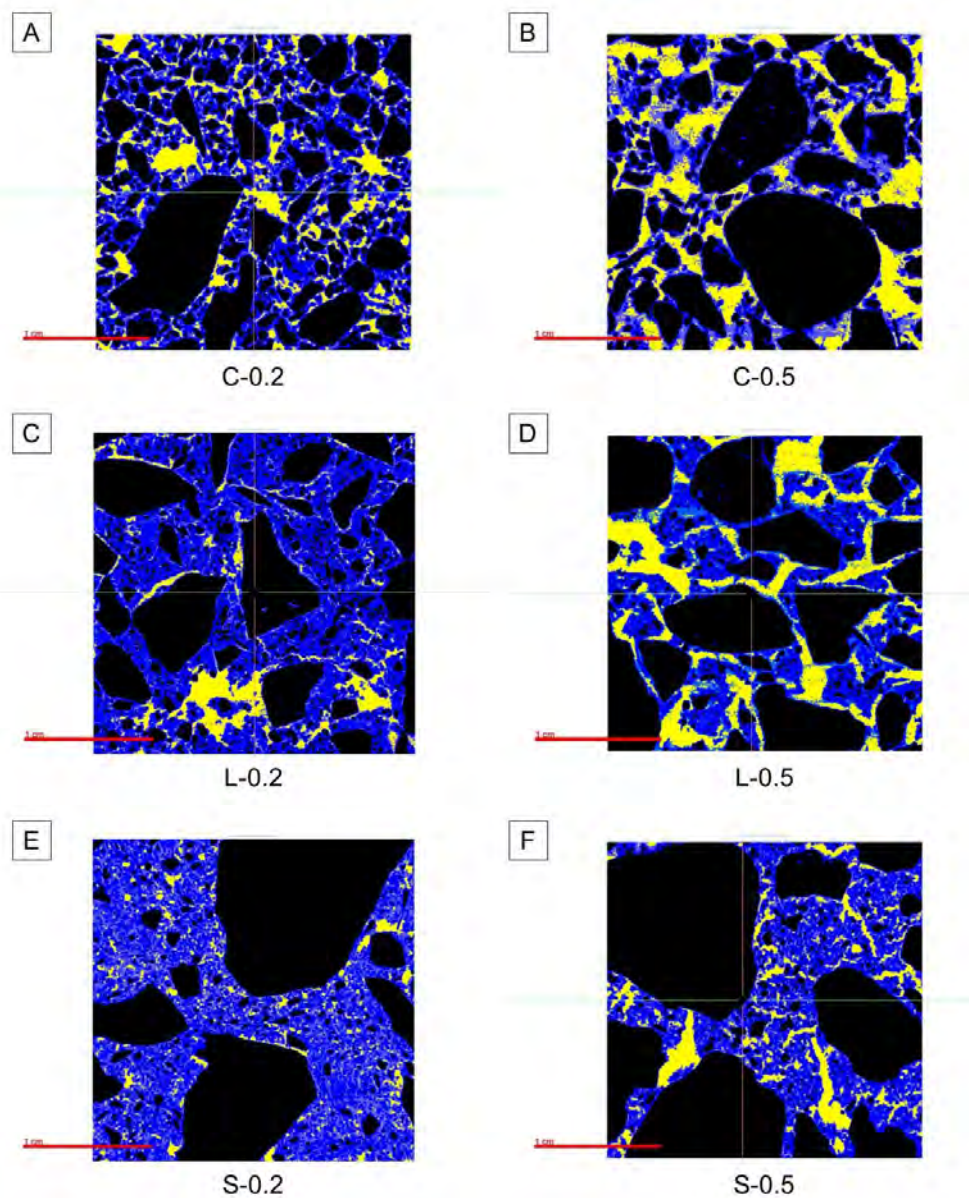


Figure 4.12: Median pore diameter (mm) of agar-based composites based on volume thickness map.

Several trends are visible in the porosity measurements obtained from X-ray tomography. Firstly, an increase in binder content led to an increase in the volume of pores (*i.e.*, comparison of 0.2 and 0.5 binder content in Figure 4.11) and pore size (*i.e.*, Figure 4.12). These results align with visual observations of composites. Agar-based composites with a 0.5 binder content had visible surface cracks concentrated between large aggregate particles (Figure 4.3). As biopolymers dehydrate, shrinkage stresses draw aggregate particles towards one another and compress the pore space of the composite (Fatehi et al. 2021). The use of too much biopolymer in a composite can result in increased internal stresses during dehydration, which can lead to particle agglomeration and a sample structure with larger, more connected voids (Hamza et al. 2023a). Porosity data suggests that a 0.5 binder content (*i.e.*, by mass) surpasses the optimal agar proportion for the aggregate and filler compositions studied herein.

Second, porosity measurements indicate that the inclusion of sand led to a decrease in the porosity of samples, evident by comparison of sand reinforced samples and control samples. The porosity of sand reinforced samples with a 0.2 and 0.5 binder content was 2.4-4.5% and 6.9-7.1% lower than comparable control samples (*i.e.*, C-0.2 and C-0.5), respectively. Conversely, the porosity of limestone and sand reinforced samples was greater than the porosity of samples with only sand (*i.e.*, porosity of LS-0.2 and LS-0.5 samples was 1.8 to 2.2% and 6.6 to 6.8% higher than S-0.2 and S-0.5 samples, respectively).

Representative 2D cross-sections from X-ray tomography reconstructions are shown in Figure 4.13. The interparticle structure of samples is illustrated using a color map of segmented sample components. Dense particles (*i.e.*, aggregate), less dense components (*i.e.*, fine particles and biopolymer), and pores are shown in black, blue, and yellow, respectively.



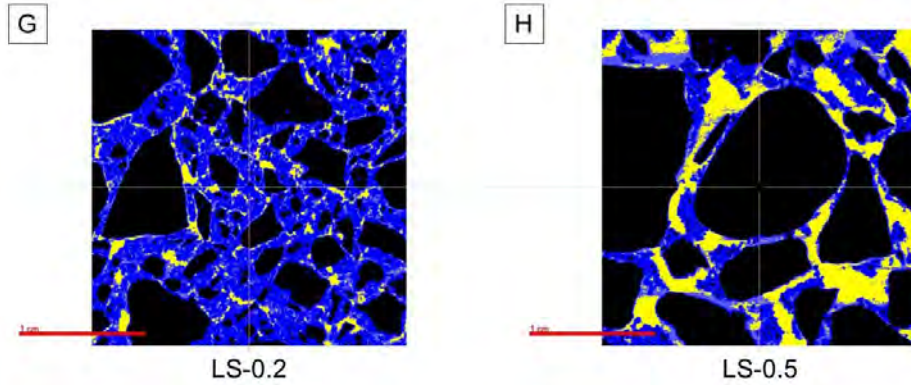


Figure 4.13: Representative cross-section of agar-based composites for A) C-0.2, B) C-0.5, C) L-0.2, D) L-0.5, E) S-0.2, F) S-0.5, G) LS-0.2, and H) LS-0.5 samples. Dense aggregate, binder and fines, and voids shown in black, blue, and yellow, respectively. Red scale bar represents 1 cm length.

Biopolymers like agar improve composite mechanical properties by forming a connected film around the surface of particles (Chang et al. 2015b, 2016; Kantesaria et al. n.d.; Muguda et al. 2017). When biopolymer composites are dehydrated, this thin film remains and functions as a matrix for particles (Chang et al. 2015c; Hamza et al. 2023a) improving interaggregate contact area (Kantesaria et al. n.d.; Smitha and Sachan 2016), sample density, and porosity (Verma et al. 2023). In a dehydrated state, mechanical improvements of biopolymer aggregate composites are primarily due to frictional forces in the sample rather than adhesion or charge interactions related to the gel strength of agar (Chang et al. 2015c).

A similar film or coating structure on the surface of aggregate particles can be observed in Figure 4.13. Sand reinforced samples demonstrated a condensed structure, evident by comparison of panels A and B with panels E and F (*i.e.*, Comparison of C-0.2 and C-0.5 with S-0.2 and S-0.5). Samples with limestone did not demonstrate the same trend. Although it appears the addition of limestone may have minimally improved the interparticle structure of control composites (*i.e.*, comparison of panels A and B with panels C and D), the interparticle structure was largely similar to comparable control composites (*i.e.*, large pore spaces are present between dense particles).

Unconfined Compressive Strength

The results for unconfined compressive strength testing of agar-based composites are shown in Figure 4.14. The compressive strength of samples ranged from 431 to 780 kPa, 500 to 913 kPa, 699 to 1,042 kPa, and 679 to 978 kPa for control, L, S, and LS samples, respectively. Compressive

strength data were further analyzed as a response variable in Scheirer Ray Hare (SRH) testing order to determine the influence of binder content and aggregate and filler composition.

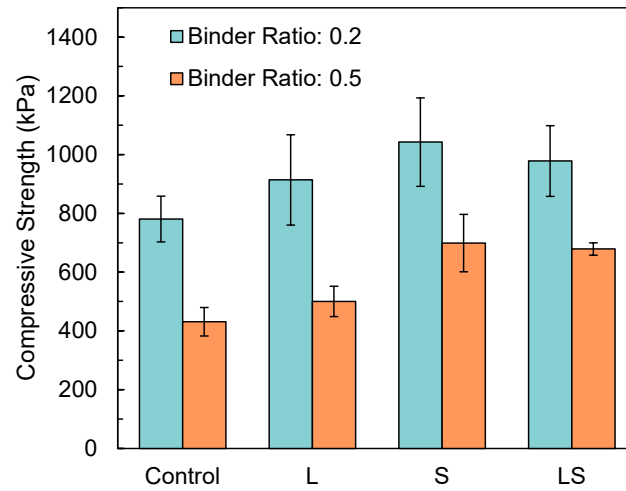


Figure 4.14: Compressive strength of agar-based composites. Error bars represent standard deviation of five samples.

The normal quantile-quantile (Q-Q) plot comparing sample quantiles and theoretical quantiles is shown in Figure 4.15. Sample quantiles illustrated significant deviation from the 1:1 diagonal line, indicating that the parametric assumptions of data normality may be violated. To account for this, nonparametric Scheirer Ray Hare (SRH) statistical testing at a 95% confidence interval was implemented to compare compressive strength results.

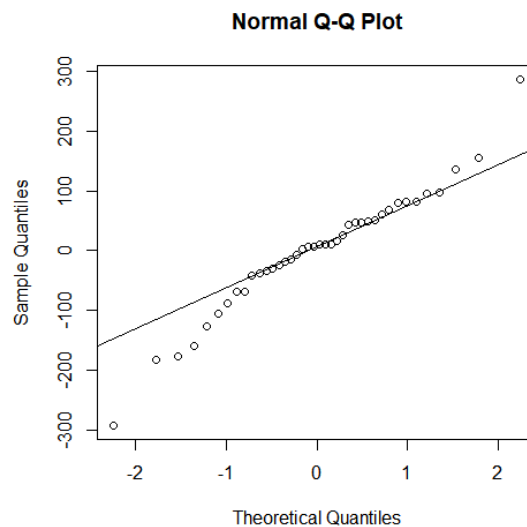


Figure 4.15: Normal quantile-quantile (Q-Q) plot for compressive strength results.

Results of SRH statistical analysis are shown below in Table 4.2. Results from this analysis indicate that both binder content ($p = 1.29\text{E-}06$) and aggregate and filler composition ($p = 0.0401$) led to statistically significant differences in composite compressive strength. The interaction between factors was not statistically significant at a 95% confidence interval (*i.e.*, p value > 0.05). A statistically significant interaction would indicate that the analyzed factors have a combined impact on the response variable that is not present in an analysis of each variable alone. These results indicate that the binder content had the same effect on compressive strength no matter the aggregate and filler composition used, and vice versa.

Table 4.2: Results for Scheirer Ray Hare analysis used to evaluate compressive strength results for agar-based composites. Results shown in bold with an asterisk (*) represent statistically significant results.

Source of Variation	df	SS	H	p-value
Binder Content	1	3,204.10	23.4446	1.29E-06*
Aggregate and Filler Composition	3	1,134.80	8.3034	0.0401*
Interaction	3	43.5	0.3183	0.9566
Residuals	32	947.6		

The use of a higher binder content in composites (*i.e.*, 0.5) led to a statistically significant decrease in composite compressive strength as compared to composites with a lower binder content (*i.e.*, 0.2). These results align with visual observations of shrinkage cracking on acclimated samples and X-ray tomography results for samples with a 0.5 binder content. To illustrate this, Figure 4.16 compares the mean compressive strength of agar-based composites with the porosity obtained in X-ray tomography processing. A linear relationship was fit to this data in order to demonstrate the relationship between these values. Similar relationships between compressive strength and porosity have been illustrated for engineering materials like pervious concrete (Barnhouse and Srubar 2016).

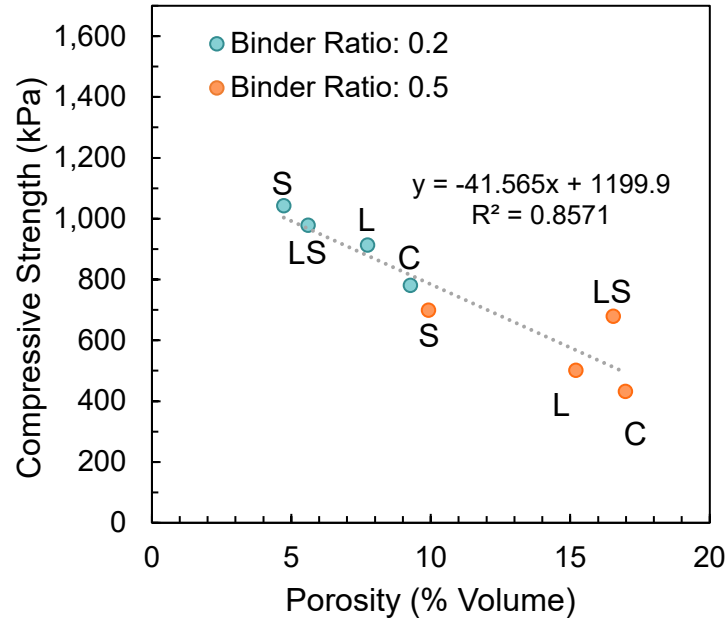


Figure 4.16: Average compressive strength (kPa, n=5) of agar-based composites potted against the porosity (% volume) of composites, as measured from x-ray tomography analysis.

Although the SRH analysis results in Table 4.2 can indicate whether statistical differences exist in a group, these results do not explicitly indicate which specific groups differ from one another. In order to determine pairwise differences, a Dunn's test was applied to compare groups in detail. As there are only two binder contents (*i.e.*, 0.2 vs 0.5 binder content) and these sample types were determined to be significantly different in SRH analysis, the Dunn's test was applied to analyze the impact of aggregate and filler composition on compressive strength. Results are shown below in Table 4.3 with significant results shown in bold and with an asterisk (*i.e.*, $p < \alpha/2$ or 0.025).

Table 4.3: Results for post hoc Dunn's analysis used to evaluate the impact of aggregate composition on the compressive strength of agar-based composites. Results shown in bold with an asterisk (*) represent statistically significant results.

Pairwise Sample Comparison	Z	p value
C-L	-0.9564	0.33888
C-LS	-2.2570	0.02401*
L-LS	-1.3007	0.19338
C-S	-2.5248	0.01158*
L-S	-1.5684	0.11678
LS-S	-0.2679	0.78887

Of the six pairwise comparisons analyzed, the compressive strength of two sample pairs were statistically significant (*i.e.*, C-LS and C-S). This indicates that the inclusion of sand in the aggregate and filler composition of samples led to a statistically significant improvement in the compressive strength of agar-based composites as compared to sample equivalents without sand (*i.e.*, control samples, or limestone reinforced). The inclusion of limestone filler reinforcement (*i.e.*, L or LS) did not significantly impact the compressive strength of agar-based composites (*i.e.*, pairwise comparisons of C-L, L-LS, and L-S generated a p value > 0.025) and composites with both limestone and sand were not found to be significantly different from samples with sand alone (*i.e.*, LS-S, p value = 0.78887). Statistical analysis indicates that unlike sand, the addition of limestone did not lead to a statistically significant impact on the compressive strength of composites.

Compressive strength results are within range of previous work conducted on biopolymers composites. Work conducted by Khatami and O’Kelly (Khatami and O’Kelly 2013) on sand treated with 1, 2, and 4% agar found that the dehydrated (*i.e.*, 30 days of acclimation) compressive strength of samples ranged from 158 to 487 kPa. Similarly, Fatehi et al. (Fatehi et al. 2021) reported unconfined compressive strengths as high as 225 kPa and 1,800 kPa for agar composites composed of sand and clays, respectively and Chang et al. (Chang et al. 2015c) reported an unconfined compressive strength of 3,190 kPa for agar and clay composites with thermal treatment. Notably, experimental results noted are for agar composites with fine particles (*i.e.*, sand or clays) and not for composites with dense-graded, coarse aggregates. The stress-strain response and area used in modulus of resilience calculations for each sample is shown in Figure 4.17 - Figure 4.24.

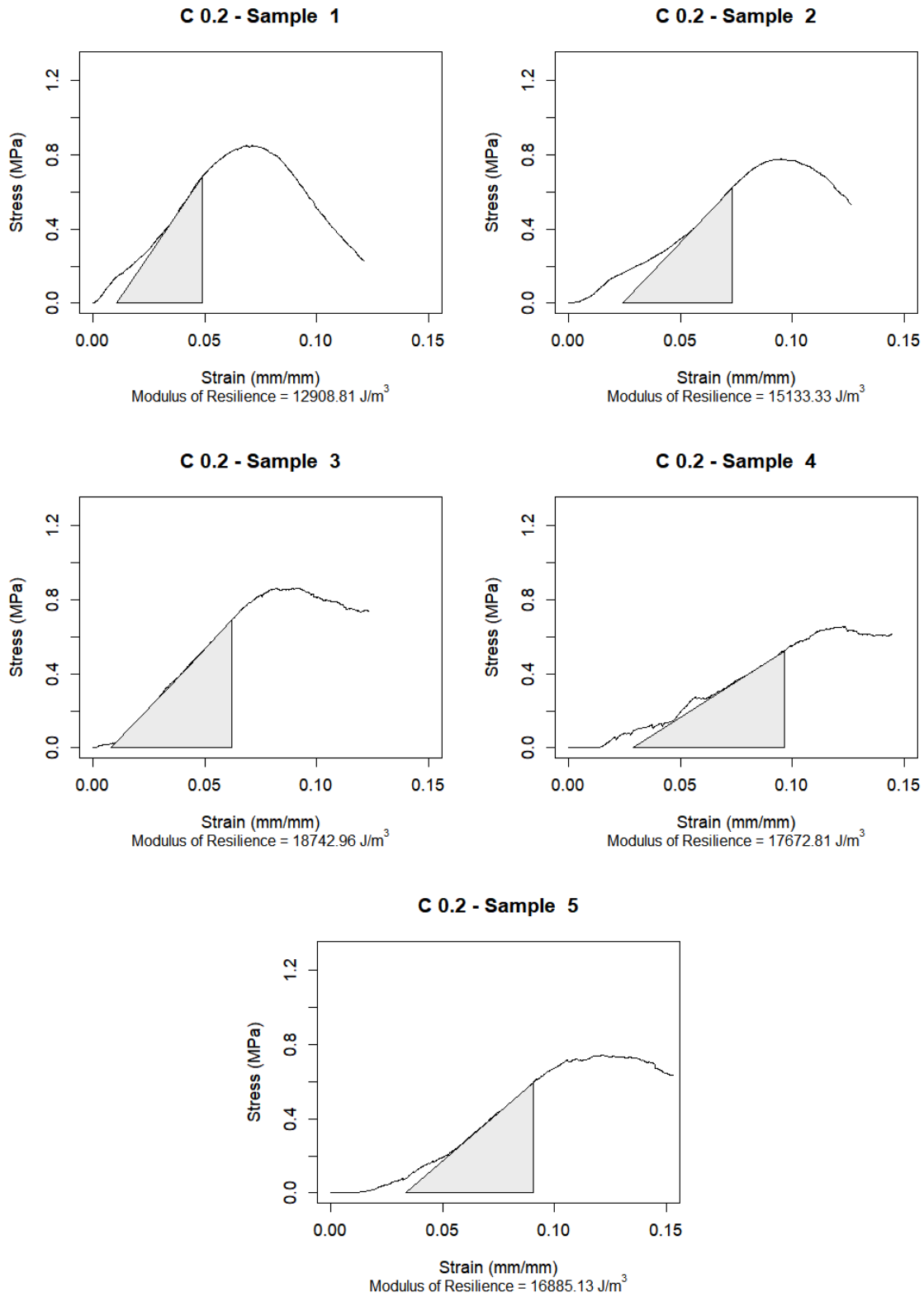


Figure 4.17: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m^3) calculations for C-0.2 samples ($n=5$).

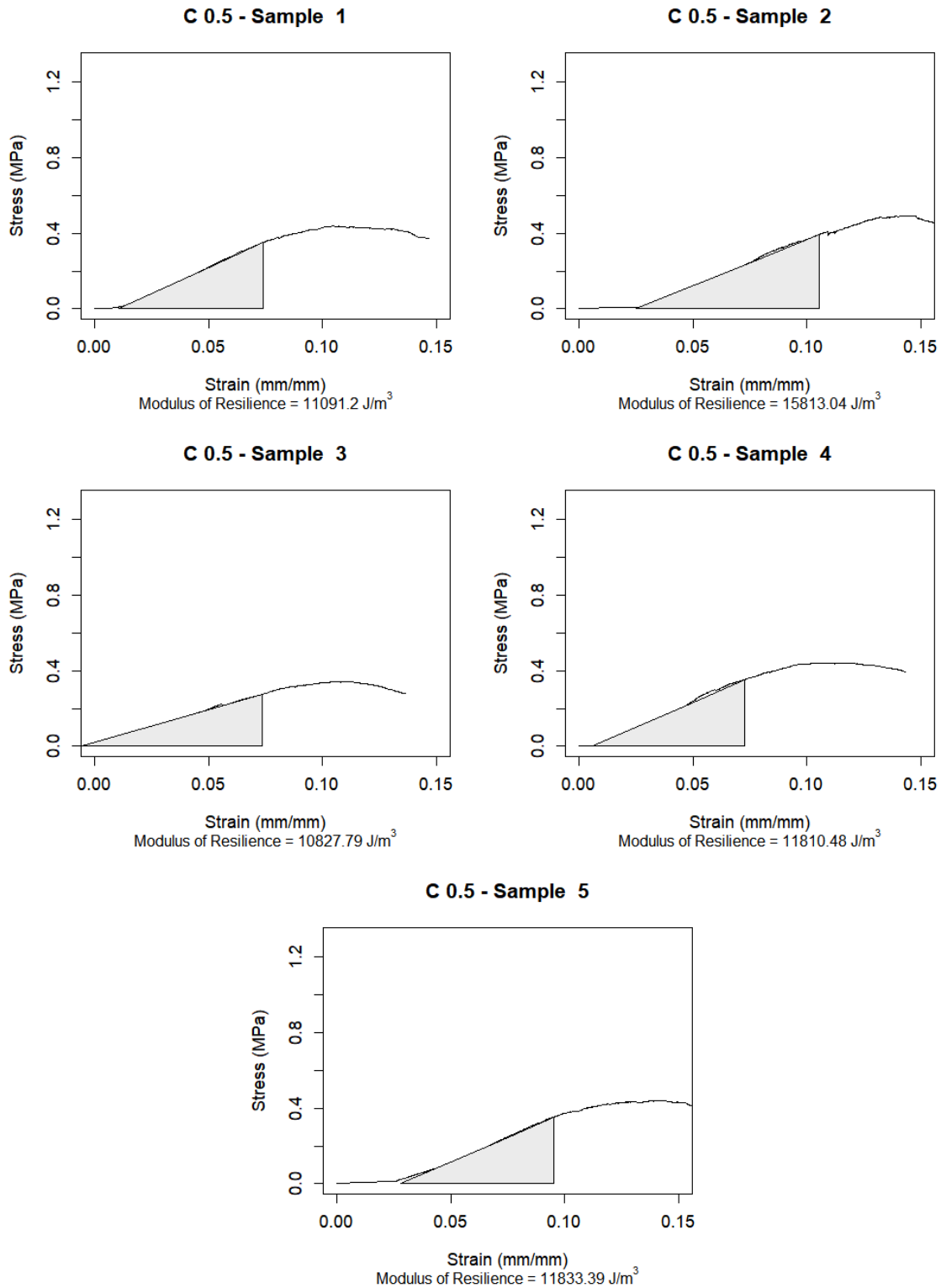


Figure 4.18: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m^3) calculations for C-0.5 samples ($n=5$).

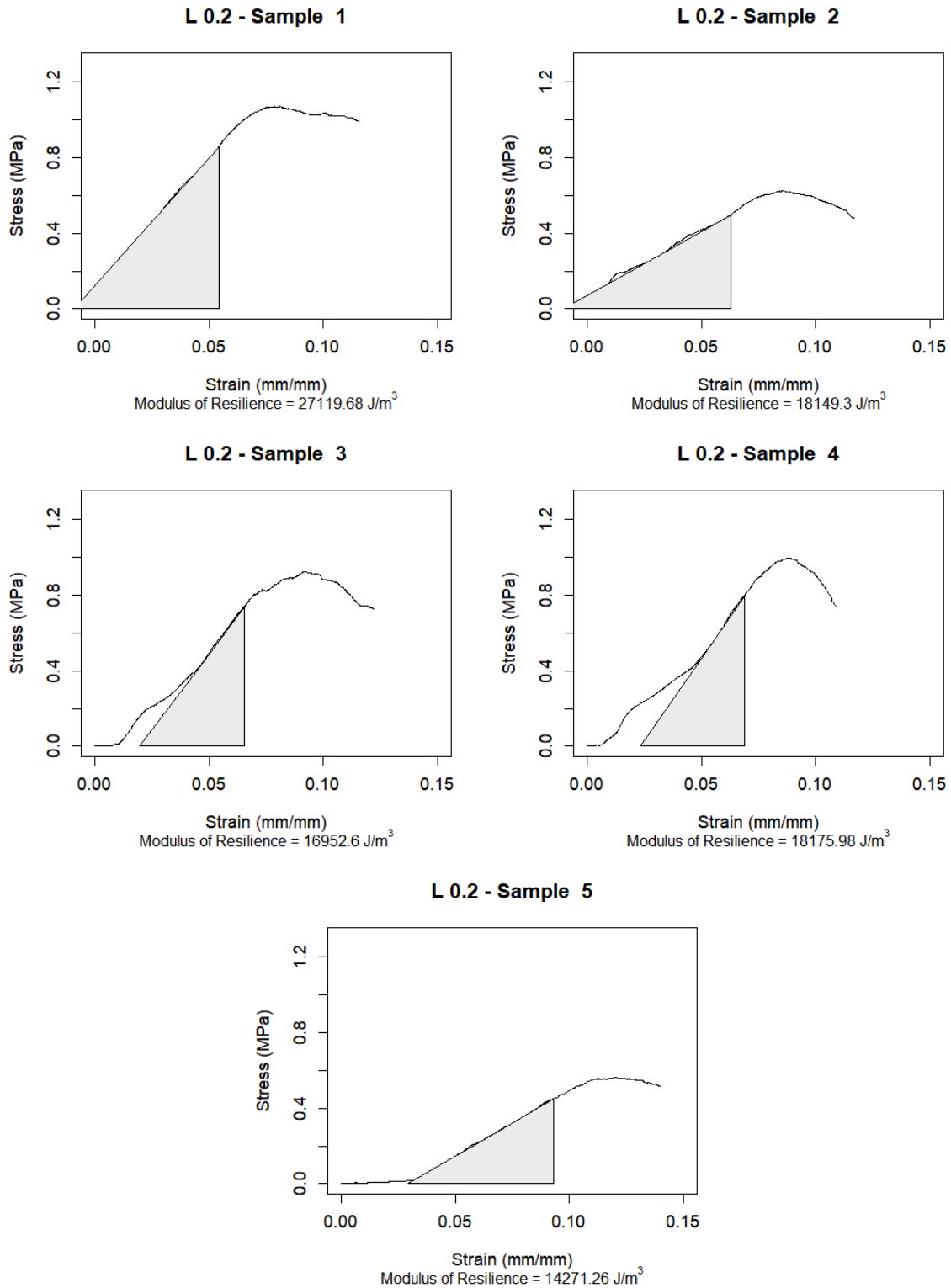


Figure 4.19: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m³) calculations for L-0.2 samples (n=5).

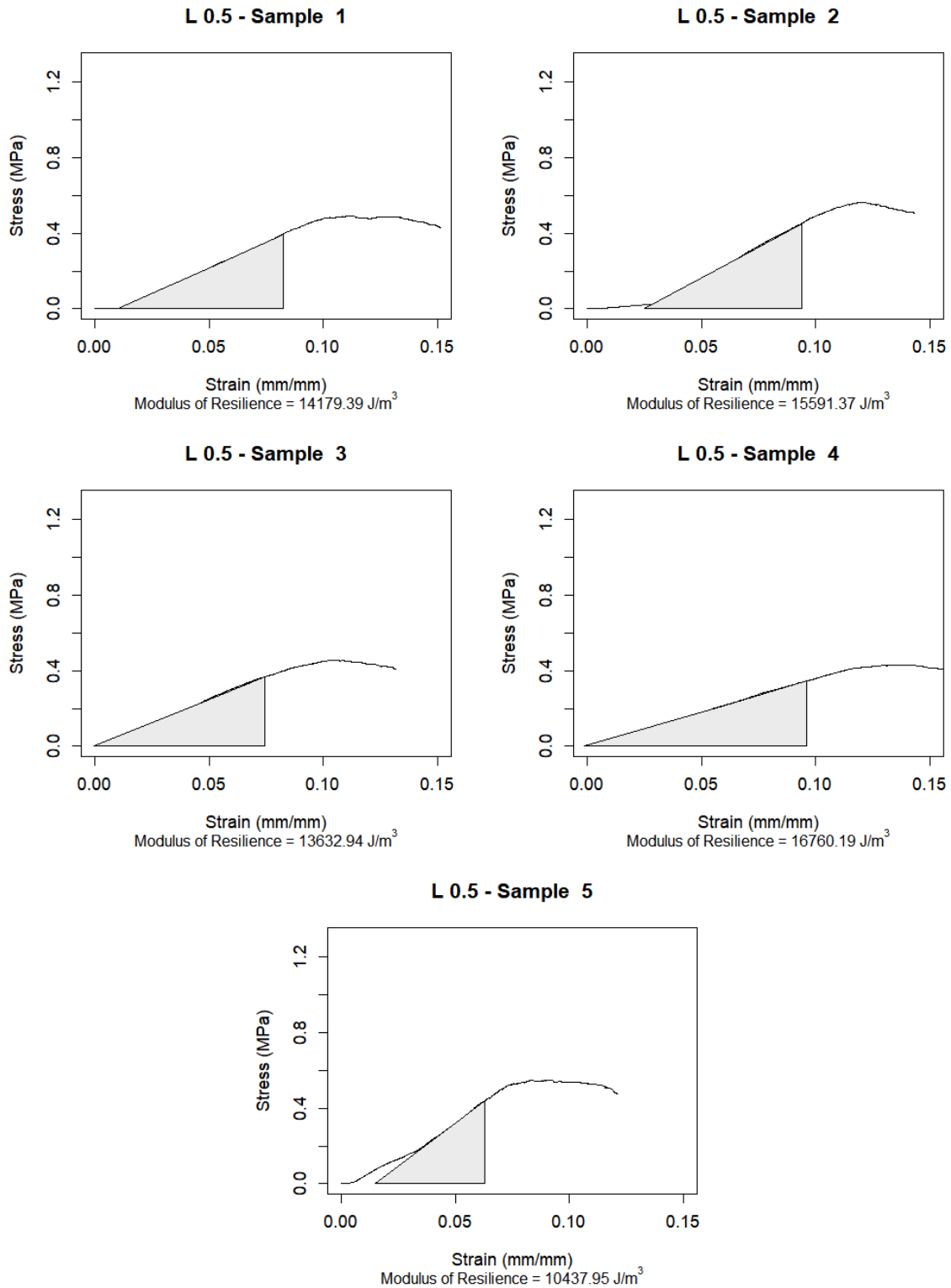


Figure 4.20: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m³) calculations for L-0.5 samples (n=5).

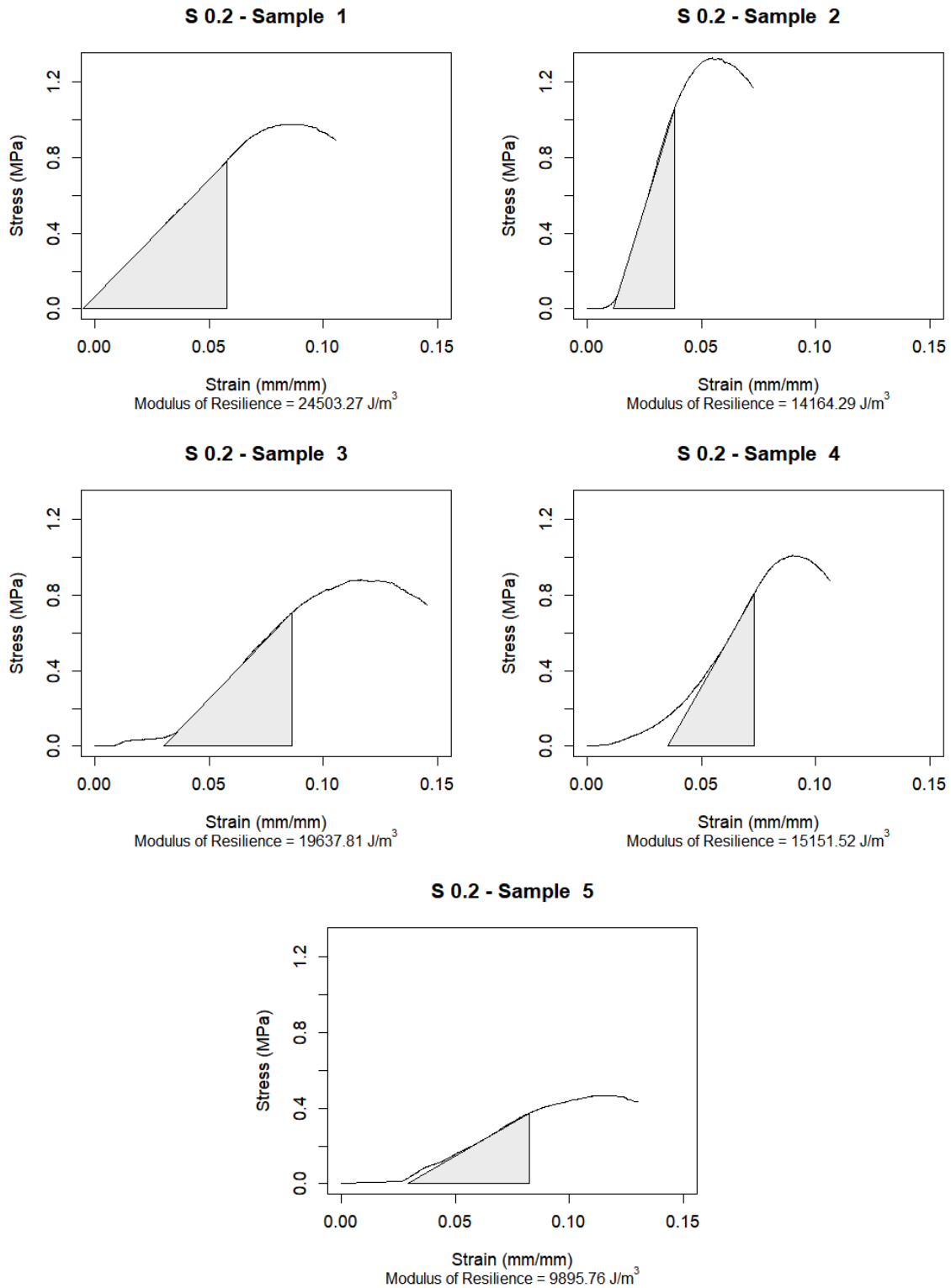


Figure 4.21: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m³) calculations for S-0.2 samples (n=5).

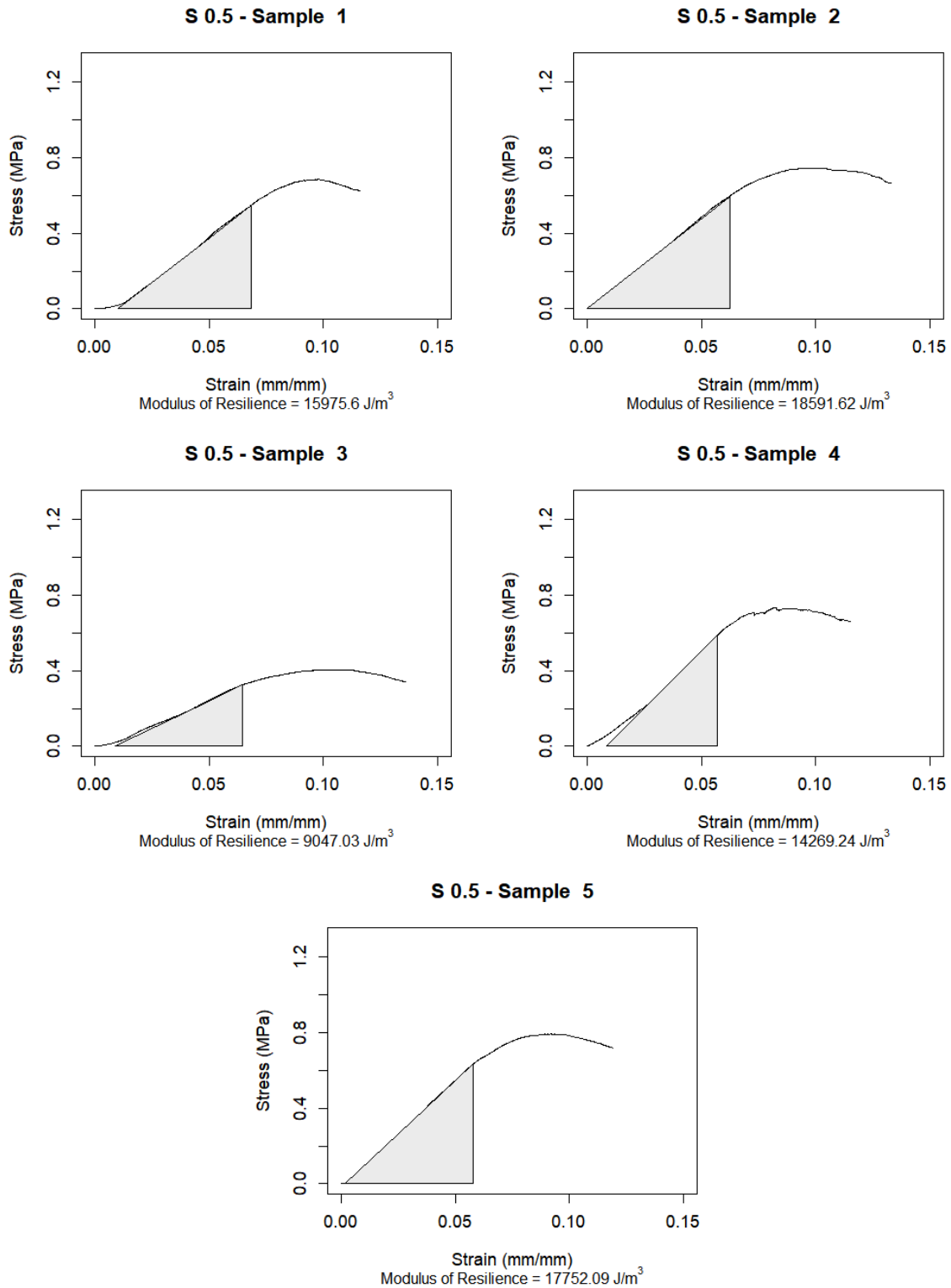


Figure 4.22: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m^3) calculations for S-0.5 samples ($n=5$).

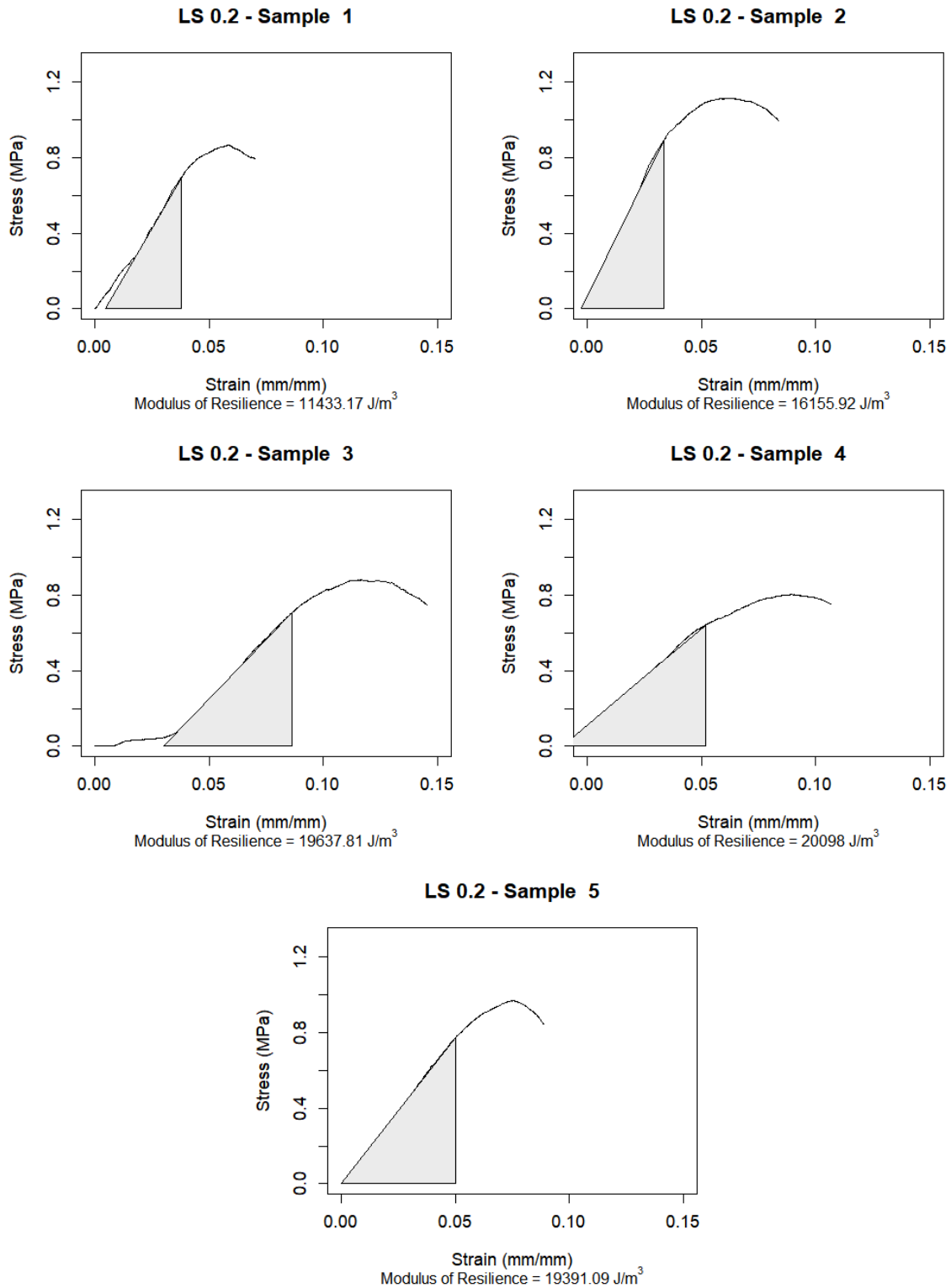


Figure 4.23: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m^3) calculations for LS-0.2 samples ($n=5$).

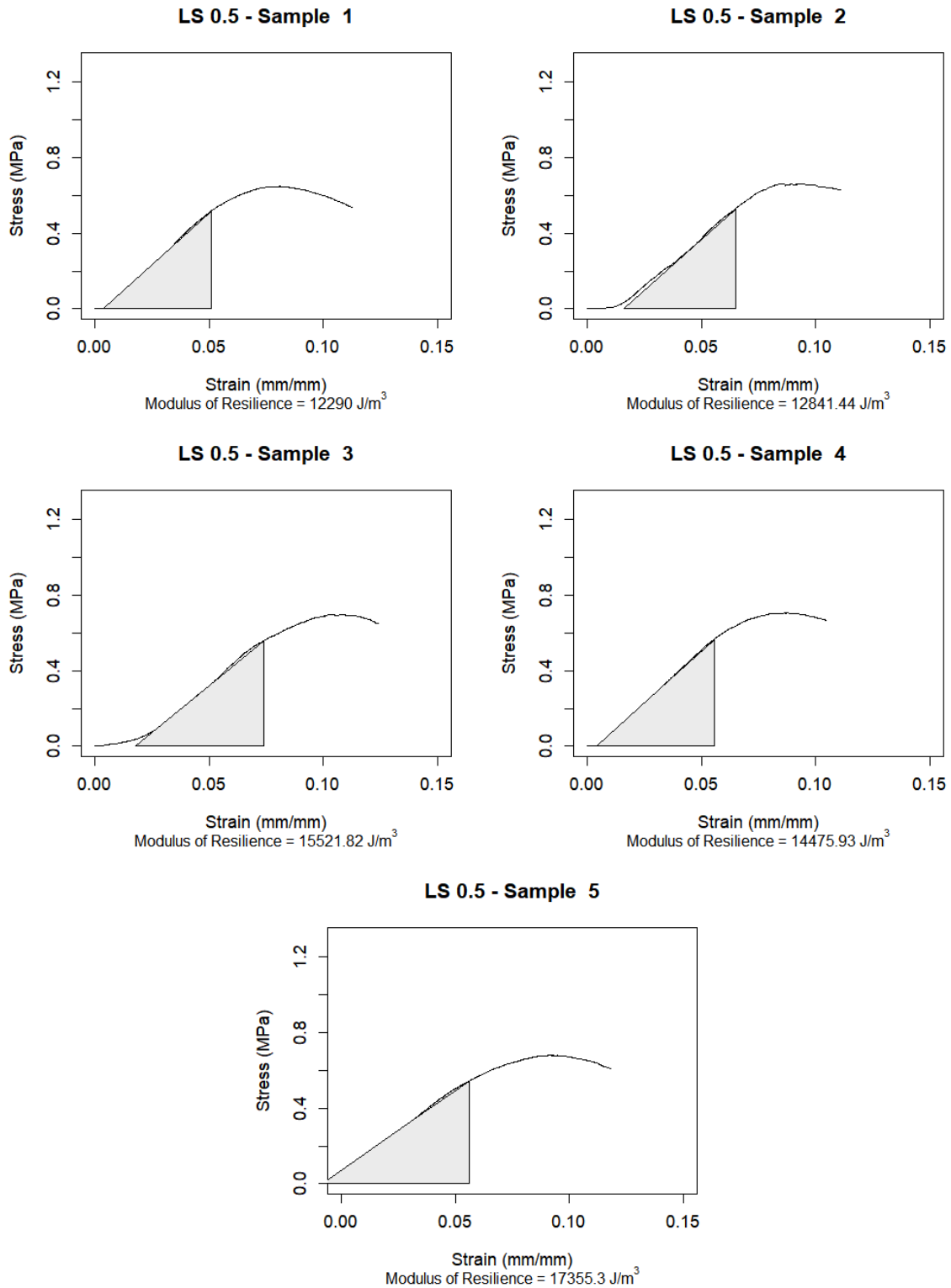


Figure 4.24: Stress (MPa) vs strain (mm/mm) curve and representative area used in the modulus of resilience (J/m^3) calculations for LS-0.2 samples ($n=5$).

Modulus of Resiliency

The moduli of resilience of agar-based composites are shown in Figure 4.25. The average modulus of resilience of samples ranged from 12,275 J/m³ (*i.e.*, sample C-0.5) to 18,934 J/m³ (*i.e.*, sample L-0.2). These results align with findings in literature. Namely, Cabalar et al. (2023) reported an increase in the modulus of resilience (*i.e.*, energy absorption capacity increased) to ~4,000 J/m³ for crushed rock stabilized with xanthan gum. Hamza et al. (2023a) found that high plastic clays stabilized with agar illustrated a modulus of resiliency over 30,000 J/m³. Results in this work are within reasonable range considering that the particle size in this work is between that of the referenced literature.

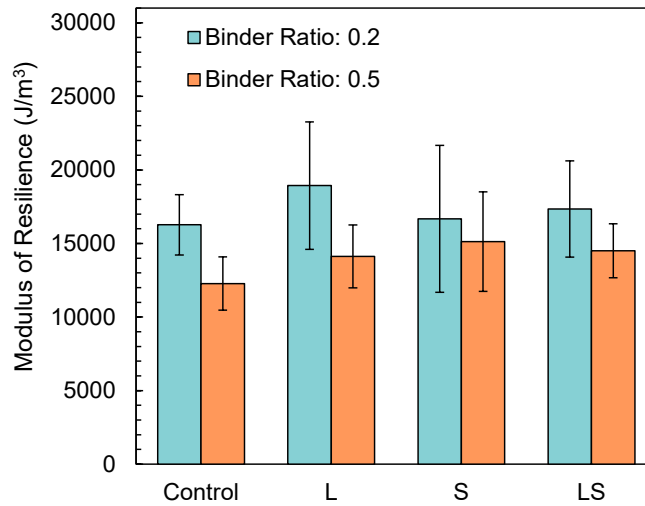


Figure 4.25: Modulus of resilience of agar-based composites. Error bars represent standard deviation of five samples.

The modulus of resilience results for agar-based composites were analyzed as a response variable in SRH testing using a 95% confidence interval in order to determine the influence of binder content and aggregate and filler composition. Results of SRH statistical analysis are shown in Table 4.4. SRH testing indicated that the aggregate and filler composition of composites did not lead to a statistically significant difference in the modulus of resilience of composites (p value = 0.70256). Similarly, the interaction between binder content and aggregate and filler composition was not statistically significant (*i.e.*, p value = 0.51246).

Table 4.4: Results for Scheirer Ray Hare analysis used to evaluate modulus of resiliency results for agar-based composites. Results shown in bold with an asterisk (*) represent statistically significant results.

Source of Variation	df	SS	H	p Value
Binder Content	1	1060.9	7.7634	0.00533*
Aggregate and Filler Composition	3	193.0	1.4127	0.70256
Interaction	3	314.3	2.3003	0.51246
Residuals (Within Group)	32	3,761.2		

Although the compressive strength of agar-based composites was significantly impacted by both experimental factors considered (i.e., binder content and the aggregate and filler composition produced a p value ≤ 0.025), the binder content was the only statistically significant factor for the modulus of resilience results (p-value = 0.00533). An increase in binder content of composites from 0.2 to 0.5 led to a 33%, 34%, 10% and 20% decrease in the average modulus of resilience for control, limestone reinforced, sand reinforced, and both limestone and sand reinforced samples, respectively.

Modulus of Elasticity

The modulus of elasticity (E) for samples is illustrated in Figure 4.26. Moduli ranged from 4.87 to 12.28, 5.91 to 12.17, 9.74 to 16.65, and 10.24 to 18.03 MPa for control, limestone reinforced, sand reinforced, and limestone and sand reinforced samples, respectively. Previous work conducted by Chang et al. (Chang et al. 2015c) found the modulus of elasticity of 1% agar-treated clayey soils to range from 188 to 270 MPa depending on thermal treatment. The magnitude of difference in moduli results in this work was expected, as the agar-based composites investigated in this work utilize much larger particles (i.e., coarse aggregate instead of sand). A material with larger particles will often reflect a smaller modulus of elasticity due to increased porosity and reduced interparticle bonding and load transfer efficiency.

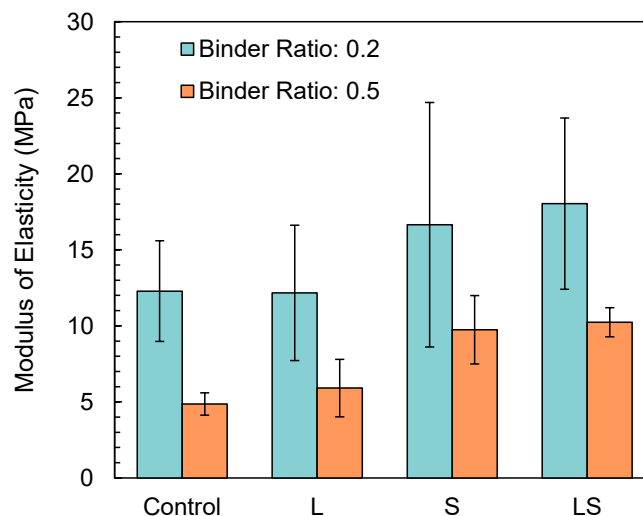


Figure 4.26: Modulus of elasticity of agar-based composites. Error bars represent standard deviation of five samples.

Results were analyzed as a response variable in SRH testing using a 95% confidence interval and results are shown in Table 4.5. Similar to the modulus of resiliency, results from this analysis indicate that only binder content ($p = 3.0E-5$) led to statistically significant differences in composite modulus of elasticity.

Table 4.5: Results for Scheirer Ray Hare analysis used to evaluate modulus of elasticity results for agar-based composites. Results shown in bold with an asterisk (*) represent statistically significant results.

Source of Variation	df	SS	H	p Value
Binder Content	1	2,371.60	17.3532	3.0E-5*
Aggregate and Filler Composition	3	965.80	7.0668	0.0698
Interaction	3	166.60	1.2190	0.74845
Residuals (Within Group)	32	1,826.00		

Environmental Impact and Cost Comparison

A literature review on the economic and environmental impacts of agar-based composites was conducted in order to understand the feasibility of using agar-based composites. It should be noted that the environmental impact of biopolymers is highly dependent on energy supply pathways, fuel sourcing, and the production method (Fatehi et al. 2021). Further, literature has noted that the novelty of industrial seaweed cultivation has resulted in a large range of estimates for

environmental and economic impacts as most of this technology has only been assessed at laboratory scale (Parsons et al. 2019).

A life cycle analysis (LCA) conducted by 3M on the production of plastic petri dishes for microbiology noted that suitable data sets were not available to estimate the impact of the agar itself. Due to the relatively low environmental impact of agar in comparison to plastic, authors chose to neglect the agar material from the LCA altogether (Howland and Bakken 2015). More recent work evaluated the cradle to gate impacts of the cultivation of *Gracilaria edulis* for the production of both seaweed extract and agar. The carbon footprint for the production of 1,000 L of extract was 73.2 kg CO₂-eq but the authors note that a majority of the impacts were associated with the use of plastics and chemicals during processing and seaweed expulsion. Notably, this work assumed a large transportation distance (100 km), the use of diesel for processing, and did not consider the CO₂ sequestered during seaweed growth (Vijay Anand et al. 2018). Parsons et al estimated that the climate change impact for single cell oils from macroalgae was 2.5 to 9.9 kg CO₂ eq per kg of oil but noted that the benefits and risks of large-scale seaweed cultivation hard to define and measure (2019).

Significant cost ranges of agar have produced uncertainty regarding large-scale production costs. This range in price is illustrated in literature- Work conducted by Vijay et al assumed a price of \$23.08 per kg of agar (2018). Conversely, Jang et al. utilized a commercial market price of \$2,000 to \$5,000 per ton of agar (\$2.21 to \$5.51 per kg) in cost estimates (2020). The lab-grade agar purchased for this work cost \$397 per kg. The authors note this variance in cost is due to differences in the material quality of biopolymer. For example, agar biopolymers for food or microbiology culture media require a higher level of purity and increased material testing (Jang 2020). Considering that about 83-90% of the current seaweed market is produced at food grade (Parsons et al. 2019) there is great potential to reduce this price with the cultivation of agar that is not food grade or sterile quality.

Utilizing the minimum (*i.e.*, \$2.21/kg) and the maximum unit cost estimated (*i.e.*, \$397/kg) the cost of agar to prepare 1 gallon of 5% w/w agar-based material could cost between \$0.42 to \$75.14. These results do not consider the cost of the water source, long term maintenance, performance, and the environmental capital required to set up new forms of manufacturing/production. For reference, Fini et al. found the cost of swine manure to be \$0.54

per gallon and estimated the cost of petroleum-based binder to be approximately \$2 per gallon (Fini et al. 2011).

This price estimate represents an exorbitant range and illustrates the largest barrier of algae-based technologies- namely the wide range of possible economic and environmental impacts. However, these results illustrate benefits of investing in carbon negative technology (*i.e.*, potential for CO₂ adsorption) and highlight the need for additional studies on the impact of agar and the best methods of economic and environmental impact reduction.

4.5 Conclusions

Microstructural and mechanical characterization was completed on agar-based composites to compare these materials with traditional engineering materials and other biopolymer composites in literature. The experimental results indicate the following:

- In microstructural evaluation, several trends are visible in porosity measurements. The porosity of agar-based composites ranged from 5.79 to 16.99% for control samples, 6.25 to 15.21% for limestone-containing samples (L), 3.39 to 9.92% for sand-containing samples (S), and 5.60 to 16.55%, for limestone and sand containing (LS) samples. An increase in binder content led to an increase in the volume of pores and pore size. The inclusion of sand led to a decrease in the porosity of samples and porosity of sand reinforced samples with a 0.2 and 0.5 binder content was 2.4-4.5% and 6.9-7.1% lower than comparable control samples (*i.e.*, C-0.2 and C-0.5), respectively. Conversely, the porosity of limestone and sand reinforced samples was greater than the porosity of samples with only sand.
- In terms of mechanical characterization, the compressive strength of samples ranged from 431 to 780 kPa, 500 to 913 kPa, 699 to 1,042 kPa, and 679 to 978 kPa for control, L, S, and LS samples, respectively. The average modulus of resilience of samples ranged from 12,275 J/m³ (*i.e.*, sample C-0.5) to 18,934 J/m³ (*i.e.*, sample L-0.2) and the average modulus of elasticity ranged from 4.87 MPa (*i.e.*, sample C-0.5) to 18.03 MPa (*i.e.*, sample LS-0.2). The use of a higher biopolymer content led to increased shrinking stresses and 0.5 binder content samples consistently illustrated lower mechanical properties.
- Economic and environmental costs of agar-based materials are difficult to evaluate as there is uncertainty regarding large-scale production costs and impacts and literature is rather

varied regarding the explicit impact of agar technologies. Although agar is currently more expensive than traditional materials, there are opportunities to reduce material cost through the cultivation of lower quality (*i.e.*, not food or microbiology oriented) agar and by optimizing processing methods.

- As shown in X-ray tomography, agar-based materials coated aggregate particles, resulting in increased sample density and frictional contact between particles. Results indicate there is likely the existence of an optimum aggregate composition and binder content. Further, X-ray tomography illustrated the formation of large fractures due to shrinkage stresses in samples with a 0.5 agar binder content. Coupled with results from porosity measurements and mechanical data, this suggests that a 0.5 binder content (*i.e.*, by mass) surpasses the optimal agar proportion for the aggregate and filler compositions studied herein.

Results from this work indicate that agar-based composites illustrate many properties in line with those of traditional engineering materials. Although the mechanical properties are lower than those of many typical engineering materials, results were in line with previous work on earthen material stabilization. Further, the porosity of agar-based composites was similar to that of pervious concrete or porous asphalt. This research is promising but highlights the need for additional work regarding optimization of binder and aggregate, the largescale environmental and economic impacts, the long-term impact of moisture on composite properties, and the evaluation of other durability mechanisms like UV.

5. Conclusions and Recommendations for Future Work

5.1 Research Summary

This research explored the potential of agar-based materials for use as direct alternatives for petroleum-based materials in pavement applications. The intention of this research was to evaluate the properties and characteristics of agar-based materials in context with civil engineering applications that are known and well established within the construction industry. Results from these studies (*i.e.*, Chapters 2, 3, and 4) demonstrated potential for agar-based materials to be used in green building materials, geotextiles, and binder applications. Although many of the results herein highlight the potential of agar-based materials, this work also highlights inherent material challenges and differences between traditional petroleum-based construction materials and agar. The contributions of this research to the state-of-the-art (*i.e.*, biopolymer research, materials science research) as well as the state-of-the-practice (*i.e.*, civil engineering research and applications) are summarized below. Additionally, the limitations of this research and recommended future work are reviewed.

5.2 Contributions of Research

Contributions to the State of the Art

The research conducted in Chapter 2 advances the knowledge of the practical engineering properties and durability of high concentration agar-based materials and allows for a side-by-side comparison of agar to a prevalent, well characterized engineering material (*i.e.*, petroleum-based asphalt binders). To authors' knowledge, no other literature has evaluated agar-based materials within the specific context of asphalt binder testing techniques. Additionally, the research in Chapter 2 enhances the scientific body of knowledge related to agar-based material degradation which can guide the future development and formulation of biopolymeric materials in civil engineering applications.

Chapter 3 evaluates and compares both chemical (*i.e.*, cross-linking with maleic anhydride) and physical (*i.e.*, the addition of inert fillers like limestone powder and sand) methods for improving agar-based material hydrophobicity. The work in this chapter enhances the body of literature related to understanding and characterizing how biopolymers like agar absorb, release, and transport moisture. Moisture is one of the primary challenges associated with utilizing

biopolymers and notably, this challenge exists in scientific fields far removed from civil engineering applications (*i.e.*, pharmaceuticals, food packaging materials). Chapter 3 provides a suite of empirical data and numerical models which can act as an empirical tool to predict the moisture behavior of agar-based materials.

Lastly, Chapter 4 advances the scientific knowledge related to the mechanical properties and structure of agar-based composites. The composition and mechanical properties of a typical dense-graded aggregate mixture combined with agar-based materials was evaluated. Results in Chapter 4 are oriented around civil engineering applications and can aid in optimizing the composition of agar-based composites to design for material properties of interest (*i.e.*, mechanical strength, resilience, dimensional stability).

Contributions to the State of the Practice

This research provided engineering specific data on agar-based materials in order to contextualize material properties. As much as possible, this work sought to bridge materials science characterization with practical civil engineering characterization and to communicate results in a comparable way. Although agar-based materials have many similarities with traditional petroleum-based materials in the civil engineering industry, there are inherent material differences that made some direct comparisons difficult or inappropriate. With the limitations of this research in mind, the findings of this project can serve as a reference for researchers, practitioners (*i.e.*, engineers), and agencies (*i.e.*, Colorado Department of Transportation) and assist in future research related to the use of biopolymers in the civil engineering industry.

5.3 Recommendations for Future Work

This research provides an initial exploration of agar-based materials. Considering the results of this work, further investigation is encouraged for target application within the construction industry. Specific recommendations for future investigations on biopolymeric or agar-based materials are summarized below.

Material Development and Improvement

Biopolymer Blending - Compared to petroleum-based polymers, biopolymers often have lower mechanical strength, dimensional stability, and durability which can limit use in load-bearing applications. To combat this, biopolymers can be tailored by blending multiple biopolymer types

to optimize intermolecular interactions, processing needs, and overall material properties. Blending of agar with other biopolymers like carrageenan, alginate, and xanthan gum is suggested for investigation.

Reinforcement Materials - The use of filler materials can improve the mechanical, moisture and durability of agar-based materials. Fairly pure reinforcement materials were used in this research (*e.g.*, limestone, well designed dense-graded aggregate) and additional reinforcement materials should be investigated. The use of widely available and environmentally friendly materials like recycled aggregate, crushed concrete, reclaimed asphalt pavement, and waste materials (*i.e.*, recycled tires, broken glass) are suggested for investigation.

Compatibility with Traditional Engineering Materials – The compatibility of agar-based materials with existing traditional civil engineering materials is suggested for investigation. Future work related to this aim should address bonding mechanisms, differential expansion and contraction, and thermal behavior of agar-based materials with materials like concrete, asphalt, soil, metals, and timber.

Aging and Durability Mechanisms

The aging mechanisms of agar-based materials as they relate to environmental factors like temperature variation (*i.e.*, freeze-thaw), ultraviolet (UV) radiation, and chemical exposure of agar-based materials requires further investigation. The aforementioned factors are highly related to the reliability, safety, and long-term structural health of civil engineering materials that agar-based materials are integrated into. Information regarding the long-term degradation from these factors is required in order to fully understand and predict the long-term performance of agar-based materials in engineering applications.

Sustainability

The sustainability of agar-based materials in civil engineering applications is not fully understood. Although the environmental impact related to growing and cultivating agar using current manufacturing methods is very low, the environmental impacts over the entire life cycle of agar-based materials (*i.e.*, use, performance, maintenance needs, end of life options, recyclability) has not been evaluated. Additionally, as large-scale implementation of agar-based materials would require investment into new manufacturing processes, future life cycle assessments of agar-based

materials should acknowledge the impacts associated with the scaling of agar cultivation (*i.e.*, the impact and economic cost of new facilities, transportation requirements).

Standardization and Scale Up

Standardization of Materials for Engineering Applications – Future work with agar-based materials in civil engineering applications requires the establishment of standardized testing protocols and quality control measures specific to agar materials. Biopolymers like agar are largely underutilized in the engineering industry due to a lack of regulatory process for characterization, mechanical testing, and durability assessment. Established testing protocols for materials like agar-based binders will aid in the credibility and acceptance of novel agar-based materials in civil engineering applications.

Scalable Manufacturing - Further investigation is required to understand scalable processing and manufacturing techniques of agar-based materials. The feasibility, commercial availability, and cost effectiveness of nonfood-grade agar materials will require collaboration between researchers, manufacturers, and practitioners (*i.e.*, engineers) in order to address the technical and economic challenges related to expanding the application of agar.

References

- “AASHTO T 27 : Standard Method of Test for Sieve Analysis of Fine and Coarse Aggregates.”
- AAT, Advanced Asphalt Technologies. 2011. NCHRP report 673: A Manual for Design of Hot-Mix Asphalt with Commentary. National Academies Press.
<https://doi.org/10.17226/14524>
- Agarwal, S., and R. K. Gupta. 2017. “Plastics in Buildings and Construction.” *Applied Plastics Engineering Handbook*, 635–649. Elsevier.
- Ahmed, R. B., and K. Hossain. 2020. “Waste cooking oil as an asphalt rejuvenator: A state-of-the-art review.” *Construction and Building Materials*, 230 (10).
<https://doi.org/10.1016/j.conbuildmat.2019.116985>.
- Al Omari, M. M. H., I. S. Rashid, N. A. Qinna, A. M. Jaber, and A. A. Badwan. 2016. “Chapter Two - Calcium Carbonate.” *Profiles of Drug Substances, Excipients and Related Methodology*, H. G. Brittain, ed., 31–132. Academic Press.
- Alfrey Jr., T., E. F. Gurnee, and W. G. Lloyd. 1966. “Diffusion in glassy polymers.” *Journal of Polymer Science Part C: Polymer Symposia*, 12 (1): 249–261.
<https://doi.org/10.1002/polc.5070120119>.
- Al-Khateeb, G. G., and K. Z. Ramadan. 2015. “Investigation of the effect of rubber on rheological properties of asphalt binders using superpave DSR.” *KSCE J Civ Eng*, 19 (1): 127–135. <https://doi.org/10.1007/s12205-012-0629-2>.
- Ameri, A., H. F. Haghshenas, and E. H. Fini. 2023. “Future Directions for Applications of Bio-Oils in the Asphalt Industry: A Step to Sequester Carbon in Roadway Infrastructure.” *Energy Fuels*, 37 (7): 4791–4815. <https://doi.org/10.1021/acs.energyfuels.2c03824>.
- Andreopoulos, A. G., and P. A. Tarantili. 2002. “Study of biopolymers as carriers for controlled release.” *Journal of Macromolecular Science, Part B*, 41 (3): 559–578.
<https://doi.org/10.1081/MB-120004353>.
- Arab, M. G., R. A. Mousa, A. R. Gabr, A. M. Azam, S. M. El-Badawy, and A. F. Hassan. 2019. “Resilient Behavior of Sodium Alginate–Treated Cohesive Soils for Pavement Applications.” *Journal of Materials in Civil Engineering*, 31 (1): 04018361. American Society of Civil Engineers. [https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0002565](https://doi.org/10.1061/(ASCE)MT.1943-5533.0002565).
- Arham, R., S. Salengke, M. Metusalach, and M. T. Mulyati. 2018. “Optimization of agar and glycerol concentration in the manufacture of edible film.” *International Food Research Journal*, 25 (5): 1845–1851.
- Armisen, R., F. Galatas, and H. S. A. Spain. 2009. “Agar.” *Handbook of Hydrocolloids*, 82–105. Woodhead Publishing.
- Asphalt institute. 2001. Superpave mix design. Lexington: Asphalt Institute.

- Asphalt Paving Association of Iowa. 2020. Asphalt Paving Design Guide. West Des Moines: Asphalt Paving Association of Iowa.
- ASTM International. 2015a. “ASTM D4402: Standard test method for viscosity determination of asphalt at elevated temperatures using a rotational viscometer.” American Society for Testing and Materials.
- ASTM International. 2015b. “ASTM D7175: Standard Test Method for Determining the Rheological Properties of Asphalt Binder Using a Dynamic Shear Rheometer.” American Society for Testing and Materials.
- ASTM International. 2016. Test Method for Determining the Flexural Creep Stiffness of Asphalt Binder Using the Bending Beam Rheometer (BBR). American Society for Testing and Materials.
- ASTM International. 2020a. “ASTM D946: Standard Specification for Penetration-Graded Asphalt Binder for Use in Pavement Construction.” American Society for Testing and Materials.
- ASTM International. 2020b. “ASTM D5: Standard Test Method for Penetration of Bituminous Materials.” American Society for Testing and Materials.
- ASTM International. 2021a. “ASTM D6373: Standard specification for performance-graded asphalt binder.” American Society for Testing and Materials.
- ASTM International. 2021b. “ASTM G21: Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi.” American Society for Testing and Materials.
- ASTM International. 2022. “ASTM D570: Standard Test Method for Water Absorption of Plastics.” American Society for Testing and Materials.
- ASTM International. n.d. ASTM D570: Test Method for Water Absorption of Plastics. American Society for Testing and Materials.
- Atlas, R. M., and T. C. Hazen. 2011. “Oil Biodegradation and Bioremediation: A Tale of the Two Worst Spills in U.S. History.” *Environ. Sci. Technol.*, 45 (16): 6709–6715. American Chemical Society. <https://doi.org/10.1021/es2013227>.
- Audo, M., M. Paraschiv, C. Queffelec, I. Louvet, J. Hemez, F. Fayon, O. Lepine, J. Legrand, M. Tazerout, E. Chailleux, and B. Bujoli. 2015. “Subcritical hydrothermal liquefaction of microalgae residues as a green route to alternative road binders.” *ACS Sustainable Chemistry and Engineering*, 3: 583–590. <https://doi.org/10.1021/acssuschemeng.5b00088>.
- Aziz, M. M. A., M. T. Rahman, Mohd. R. Hainin, and W. A. W. A. Bakar. 2015. “An overview on alternative binders for flexible pavement.” *Construction and Building Materials*, 84: 315–319. <https://doi.org/10.1016/j.conbuildmat.2015.03.068>.

- Balart, R., D. Garcia-Garcia, V. Fombuena, L. Quiles-Carrillo, and M. P. Arrieta. 2021. "Biopolymers from Natural Resources." *Polymers*, 13 (15): 2532. Multidisciplinary Digital Publishing Institute. <https://doi.org/10.3390/polym13152532>.
- Barghini, A., V. I. Ivanova, S. H. Imam, and E. Chiellini. 2010. "Poly-(ϵ -caprolactone) (PCL) and poly(hydroxy-butyrate) (PHB) blends containing seaweed fibers: Morphology and thermal-mechanical properties." *J. Polym. Sci. A Polym. Chem.*, 48 (23): 5282–5288. <https://doi.org/10.1002/pola.24327>.
- Barnhouse, P. W., and W. V. Srubar. 2016. "Material characterization and hydraulic conductivity modeling of macroporous recycled-aggregate pervious concrete." *Construction and Building Materials*, 110: 89–97. <https://doi.org/10.1016/j.conbuildmat.2016.02.014>.
- Berens, A. R., and H. B. Hopfenberg. 1978. "Diffusion and relaxation in glassy polymer powders: 2. Separation of diffusion and relaxation parameters." *Polymer*, 19 (5): 489–496. [https://doi.org/10.1016/0032-3861\(78\)90269-0](https://doi.org/10.1016/0032-3861(78)90269-0).
- Bernaerts, T. M. M., L. Gheysen, I. Foubert, M. E. Hendrickx, and A. M. Van Loey. 2019. "The potential of microalgae and their biopolymers as structuring ingredients in food: A review." *Biotechnology Advances*, 37 (8): 107419. <https://doi.org/10.1016/j.biotechadv.2019.107419>.
- Billington, S. L., W. V. Srubar, A. T. Michel, and S. A. Miller. 2014. "Renewable Biobased Composites for Civil Engineering Applications." *Sustainable Composites: Fibers, Resins, and Applications*, 313–348. DEStech Publications, Inc.
- Bonati, A., F. Merusi, G. Polacco, S. Filippi, and F. Giuliani. 2012. "Ignitability and thermal stability of asphalt binders and mastics for flexible pavements in highway tunnels." *Construction and Building Materials, Non Destructive Techniques for Assessment of Concrete*, 37: 660–668. <https://doi.org/10.1016/j.conbuildmat.2012.07.096>.
- Bozyigit, I., H. O. Zingil, and S. Altun. 2023. "Performance of eco-friendly polymers for soil stabilization and their resistance to freeze–thaw action." *Construction and Building Materials*, 379: 131133. <https://doi.org/10.1016/j.conbuildmat.2023.131133>.
- C01 Committee. n.d. ASTM C109: Test Method for Compressive Strength of Hydraulic Cement Mortars (Using 2-in. or [50-mm] Cube Specimens). ASTM International.
- C09 Committee. n.d. ASTM C642: Test Method for Density, Absorption, and Voids in Hardened Concrete. ASTM International.
- Cabalar, A. F., N. Akbulut, S. Demir, and O. Yildiz. 2023. "Use of a Biopolymer for Road Pavement Subgrade." *Sustainability*, 15 (10): 8231. <https://doi.org/10.3390/su15108231>.
- Chang, I., J. Im, and G.-C. Cho. 2016. "Geotechnical engineering behaviors of gellan gum biopolymer treated sand." *Can. Geotech. J.*, 53 (10): 1658–1670. NRC Research Press. <https://doi.org/10.1139/cgj-2015-0475>.

- Chang, I., M. Lee, A. T. P. Tran, S. Lee, Y.-M. Kwon, J. Im, and G.-C. Cho. 2020. "Review on biopolymer-based soil treatment (BPST) technology in geotechnical engineering practices." *Transportation Geotechnics*, 24: 100385. <https://doi.org/10.1016/j.trgeo.2020.100385>.
- Chang, I., A. K. Prasadhi, J. Im, and G.-C. Cho. 2015a. "Soil strengthening using thermo-gelation biopolymers." *Construction and Building Materials*, 77: 430–438. <https://doi.org/10.1016/j.conbuildmat.2014.12.116>.
- Chang, I., A. K. Prasadhi, J. Im, and G.-C. Cho. 2015b. "Soil strengthening using thermo-gelation biopolymers." *Construction and Building Materials*, 77: 430–438. <https://doi.org/10.1016/j.conbuildmat.2014.12.116>.
- Chang, I., A. K. Prasadhi, J. Im, and G.-C. Cho. 2015c. "Soil strengthening using thermo-gelation biopolymers." *Construction and Building Materials*, 77: 430–438. <https://doi.org/10.1016/j.conbuildmat.2014.12.116>.
- "Chapter 3. Natural Polysaccharide-Based Hydrogels and Nanomaterials: Recent Trends and Their Applications | Elsevier Enhanced Reader." n.d. Accessed January 2, 2023. <https://reader.elsevier.com/reader/sd/pii/B9780128133514000031?token=7B2915C6C1B770C3CDB19BB4C748FF3EF2624D88E7AA4E5E74A361590DFC3FEE50305FCDA498B3282A6518D7B11D71BD&originRegion=us-east-1&originCreation=20230102214837>.
- Chen, J.-S., and C. H. Yang. 2020. "Porous asphalt concrete: A review of design, construction, performance and maintenance." *Int. J. Pavement Res. Technol.*, 13 (6): 601–612. <https://doi.org/10.1007/s42947-020-0039-7>.
- Chen, M., B. Leng, S. Wu, and Y. Sang. 2014. "Physical, Chemical, and Rheological Properties of Waste Edible Vegetable Oil Rejuvenated Asphalt Binders." *Construction and Building Materials*, 66: 286–298. <https://doi.org/10.1016/j.conbuildmat.2014.05.033>.
- Chen, R., I. Lee, and L. Zhang. 2015. "Biopolymer Stabilization of Mine Tailings for Dust Control." *Journal of Geotechnical and Geoenvironmental Engineering*, 141 (2): 04014100. American Society of Civil Engineers. [https://doi.org/10.1061/\(ASCE\)GT.1943-5606.0001240](https://doi.org/10.1061/(ASCE)GT.1943-5606.0001240).
- Chen, Z., J. Yi, Z. Chen, and D. Feng. 2019. "Properties of asphalt binder modified by corn stalk fiber." *Construction and Building Materials*, 212: 225–235. <https://doi.org/10.1016/j.conbuildmat.2019.03.329>.
- Cheraghian, G., A. Cannone Falchetto, Z. You, S. Chen, Y. S. Kim, J. Westerhoff, K. H. Moon, and M. P. Wistuba. 2020. "Warm mix asphalt technology: An up to date review." *Journal of Cleaner Production*, 268: 122128. <https://doi.org/10.1016/j.jclepro.2020.122128>.
- Chocron, S., A. Carpenter, A. Nicholls, J. Walker, O. Elbaz, and E. Rejovitzky. 2021. "Characterization of Ottawa Sand and Application to Blast Simulations." *EPJ Web Conf.*,

- (F. Gálvez Díaz-Rubio and D. A. Cendón Franco, eds.), 250: 02009.
<https://doi.org/10.1051/epjconf/202125002009>.
- Christian, S. J., and S. L. Billington. 2011. “Mechanical response of PHB- and cellulose acetate natural fiber-reinforced composites for construction applications.” *Composites Part B: Engineering*, 42 (7): 1920–1928. <https://doi.org/10.1016/j.compositesb.2011.05.039>.
- Chudasama, N. A., R. A. Sequeira, K. Moradiya, and K. Prasad. 2021. “Seaweed Polysaccharide Based Products and Materials: An Assessment on Their Production from a Sustainability Point of View.” *Molecules*, 26 (9): 2608. <https://doi.org/10.3390/molecules26092608>.
- Craig, R. R. 2000. “Stress and Strain; Design.” *Mechanics of materials*, 36–43. New York: Wiley.
- Cui, P., G. Schito, and Q. Cui. 2020. “VOC Emissions from asphalt pavement and health risks to construction workers.” *Journal of Cleaner Production*, 244 (20).
<https://doi.org/10.1016/j.jclepro.2019.118757>.
- Cunningham, C. N., T. M. Evans, and A. A. Tayebali. 2013. “Gradation effects on the mechanical response of crushed stone aggregate.” *International Journal of Pavement Engineering*, 14 (3): 231–241. Taylor & Francis.
<https://doi.org/10.1080/10298436.2012.690518>.
- D04 Committee. n.d. ASTM D75: Practice for Sampling Aggregates. ASTM International.
- D04 Committee. n.d. ASTM D7063: Test Method for Effective Porosity and Effective Air Voids of Compacted Asphalt Mixture Samples. ASTM International.
- Dave, P. N., and A. Gor. 2018. “Natural Polysaccharide-Based Hydrogels and Nanomaterials.” *Handbook of Nanomaterials for Industrial Applications*, 36–66. Elsevier.
- De Kee, D., Q. Liu, and J. Hinestroza. 2008. “Viscoelastic (Non-Fickian) Diffusion.” *Can. J. Chem. Eng.*, 83 (6): 913–929. <https://doi.org/10.1002/cjce.5450830601>.
- Dessouky, S., A. Pothuganti, L. Walubita, and D. Rand. 2013. “Laboratory evaluation of the workability and compactability of asphaltic materials prior to road construction.” *Journal of Materials in Civil Engineering*, 25 (6): 810–818.
[https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0000551](https://doi.org/10.1061/(ASCE)MT.1943-5533.0000551).
- El-hefian, E. A., M. M. Nasef, and A. H. Yahaya. 2012. “Preparation and Characterization of Chitosan/Agar Blended Films: Part 1. Chemical Structure and Morphology.” *E-Journal of Chemistry*, 9 (3): 1431–1439. <https://doi.org/10.1155/2012/781206>.
- Elkashef, M., J. Podolsky, C. R. Williams, and E. Cochran. 2017. “Preliminary Examination of Soybean Oil Derived Material as a Potential Rejuvenator through Superpave Criteria and Asphalt Bitumen Rheology.” *Construction and Building Materials*, 149: 826–836.
<https://doi.org/10.1016/j.conbuildmat.2017.05.195>.

- Enderus, N. F., and S. M. Tahir. 2017. "Green waste cooking oil-based rigid polyurethane foam." *IOP Conf. Ser.: Mater. Sci. Eng.*, 271: 012062. <https://doi.org/10.1088/1757-899X/271/1/012062>.
- Eneh, O. C. 2011. "A Review on Petroleum: Source, Uses, Processing, Products, and the Environment." *Journal of Applied Sciences*, 11 (12): 2084–2091. <https://doi.org/10.3923/jas.2011.2084.2091>.
- Engelsmann, S., S. Peters, J. Reisenberger, and V. Spalding. 2012. *Plastics: in Architecture and Construction*. Basel: Birkhäuser.
- Erdoğan, S. T., A. M. Forster, P. E. Stutzman, and E. J. Garboczi. 2017. "Particle-based characterization of Ottawa sand: Shape, size, mineralogy, and elastic moduli." *Cement and Concrete Composites*, 83: 36–44. <https://doi.org/10.1016/j.cemconcomp.2017.07.003>.
- Fang, M., D. Park, J. L. Singuranayo, H. Chen, and Y. Li. 2019. "Aggregate gradation theory, design and its impact on asphalt pavement performance: a review." *International Journal of Pavement Engineering*, 20 (12): 1408–1424. <https://doi.org/10.1080/10298436.2018.1430365>.
- Fatehi, H., D. E. L. Ong, J. Yu, and I. Chang. 2021. "Biopolymers as Green Binders for Soil Improvement in Geotechnical Applications: A Review." *Geosciences*, 11 (7): 291. Multidisciplinary Digital Publishing Institute. <https://doi.org/10.3390/geosciences11070291>.
- Federal Highway Administration. 2021. *FHWA Forecasts of Vehicle Miles Traveled*.
- Federal Highway Administration, and National Asphalt Pavement Association. 2001. *HMA Pavement Mix Type Selection Guide*.
- Fernandez, E., D. Lopez, C. Mijangos, M. Duskova-Smrckova, M. Ilavsky, and K. Dusek. n.d. "Rheological and thermal properties of agarose aqueous solutions and hydrogels." *Journal of Polymer Science Part B: Polymer Physics*, 46 (3): 322–328.
- Fini, E. H., I. L. Al-Qadi, Z. You, B. Zada, and J. Mills-Beale. 2012. "Partial replacement of asphalt binder with bio-binder: characterisation and modification." *International Journal of Pavement Engineering*, 13 (6): 515–522. <https://doi.org/10.1080/10298436.2011.596937>.
- Fini, E., E. Kalberer, A. Shahbazi, M. Basti, Z. You, H. Ozer, and Q. Aurangzeb. 2011. "Chemical Characterization of biobinder from swine manure: sustainable modifier for asphalt binder." *Journal of Materials in Civil Engineering*, 23 (11): 1506–1513. [https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0000237](https://doi.org/10.1061/(ASCE)MT.1943-5533.0000237).
- Flórez, M., P. Cazón, and M. Vázquez. 2023. "Selected Biopolymers' Processing and Their Applications: A Review." *Polymers*, 15 (3): 641. Multidisciplinary Digital Publishing Institute. <https://doi.org/10.3390/polym15030641>.

- Folino, A., A. Karageorgiou, P. S. Calabrò, and D. Komilis. 2020. "Biodegradation of Wasted Bioplastics in Natural and Industrial Environments: A Review." *Sustainability*, 12 (15): 6030. <https://doi.org/10.3390/su12156030>.
- Foster, K. E. O., R. Jones, G. M. Miyake, and W. V. Srubar. 2021. "Mechanics, optics, and thermodynamics of water transport in chemically modified transparent wood composites." *Composites Science and Technology*, 208: 108737. <https://doi.org/10.1016/j.compscitech.2021.108737>.
- Frazier, S. D., M. G. Matar, J. Osio-Norgaard, A. N. Aday, E. A. Delesky, and W. V. Srubar. 2020. "Inhibiting Freeze-Thaw Damage in Cement Paste and Concrete by Mimicking Nature's Antifreeze." *Cell Reports Physical Science*, 1 (6): 100060. <https://doi.org/10.1016/j.xcrp.2020.100060>.
- Freile-Pelegrín, Y., and T. J. Madera-Santana. 2017. "Chapter 18 - Characterization Techniques for Algae-Based Materials." *Algae Based Polymers, Blends, and Composites*, K. M. Zia, M. Zuber, and M. Ali, eds., 649–670. Elsevier.
- Ganji, F., S. Vasheghani Farahani, and E. Vasheghani-Farahani. 2010. "Theoretical Description of Hydrogel Swelling: A Review." *Iranian Polymer Journal*, 19: 375–398.
- Gao, J., H. Wang, C. Liu, D. Ge, Z. You, and M. Yu. 2020. "High-temperature rheological behavior and fatigue performance of lignin modified asphalt binder." *Construction and Building Materials*, 230: 117063. <https://doi.org/10.1016/j.conbuildmat.2019.117063>.
- George, A., M. R. Sanjay, R. Srisuk, J. Parameswaranpillai, and S. Siengchin. 2020. "A comprehensive review on chemical properties and applications of biopolymers and their composites." *International Journal of Biological Macromolecules*, 154: 329–338. <https://doi.org/10.1016/j.ijbiomac.2020.03.120>.
- Gere, J. M., and B. J. Goodno. 2013. "Axially Loaded Members- Strain Energy." *Mechanics of materials*, 180–190. Stamford: Cengage Learning.
- Geyer, R., J. R. Jambeck, and K. L. Law. 2017. "Production, use, and fate of all plastics ever made." *Sci. Adv.*, 3 (7): e1700782. <https://doi.org/10.1126/sciadv.1700782>.
- Ghabchi, R., and M. P. Pereira Castro. 2021. "Characterisation of a hybrid plant-based asphalt binder replacement with high reactive phenolic monomer content." *International Journal of Pavement Engineering*, 1–22. <https://doi.org/10.1080/10298436.2021.1969570>.
- Ghebremedhin, M., S. Seiffert, and T. A. Vilgis. 2021. "Physics of agarose fluid gels: Rheological properties and microstructure." *Curr Res Food Sci*, 4: 436–448. <https://doi.org/10.1016/j.crfs.2021.06.003>.
- Global Energy and CO2 Status Report. 2018. International Energy Agency.
- Gualtieri, P., and L. Barsanti. 2022. *Algae: Anatomy, Biochemistry, and Biotechnology*. Boca Raton: CRC Press.

- Guerrero, P., A. Etxabide, I. Leceta, M. Peñalba, and K. de la Caba. 2014. "Extraction of agar from *Gelidium sesquipedale* (Rhodophyta) and surface characterization of agar based films." *Carbohydrate Polymers*, 99: 491–498. <https://doi.org/10.1016/j.carbpol.2013.08.049>.
- Gurgel Adeodato Vieira, M., M. Altenhofen da Silva, L. Oliveira dos Santos, and M. Masumi Beppu. 2011. "Natural-based plasticizer and biopolymer films: A review." *European Polymer Journal*, 47 (3): 254–263. <https://doi.org/10.1016/j.eurpolymj.2010.12.011>.
- Hamza, M., Z. Nie, M. Aziz, N. Ijaz, O. Akram, C. Fang, M. U. Ghani, Z. Ijaz, S. Noshin, and M. F. Madni. 2023a. "Geotechnical behavior of high-plastic clays treated with biopolymer: macro–micro-study." *Environ Earth Sci*, 82 (3): 91. <https://doi.org/10.1007/s12665-023-10760-2>.
- Hamza, M., Z. Nie, M. Aziz, N. Ijaz, M. F. Ameer, and Z. Ijaz. 2023b. "Geotechnical properties of problematic expansive subgrade stabilized with xanthan gum biopolymer." *Road Materials and Pavement Design*, 24 (7): 1869–1883. <https://doi.org/10.1080/14680629.2022.2092027>.
- Han, Z., A. Sha, Z. Tong, Z. Liu, J. Gao, X. Zou, and D. Yuan. 2017. "Study on the optimum rice husk ash content added in asphalt binder and its modification with bio-oil." *Construction and Building Materials*, 147: 776–789. <https://doi.org/10.1016/j.conbuildmat.2017.05.004>.
- Hanz, A. J., and H. U. Bahia. 2013. "Asphalt Binder Contribution to Mixture Workability and Application of Asphalt Lubricity Test to Estimate Compactability Temperatures for Warm-Mix Asphalt." *Transportation Research Record*, 2371 (1): 87–95. <https://doi.org/10.3141/2371-10>.
- He, M., C. Tu, D. W. Cao, and Y. J. Chen. 2019. "Comparative analysis of bio-binder properties derived from different sources." *International Journal of Pavement Engineering*, 20 (7): 792–800. <https://doi.org/10.1080/10298436.2017.1347434>.
- Hess, K. M., and W. V. Sruhar. 2016. "Activating relaxation-controlled diffusion mechanisms for tailored moisture resistance of gelatin-based bioadhesives for engineered wood products." *Composites Part A: Applied Science and Manufacturing*, 84: 435–441. <https://doi.org/10.1016/j.compositesa.2016.02.013>.
- Hossain, M. S., and W. S. Kim. 2014. Estimation of subgrade resilient modulus using the unconfined compression test.
- Howland, J., and H. Bakken. 2015. Reduction in Primary Energy Demand, Blue Water Consumption and GHG emissions from 3M petrifil plates compared to Traditional Microbiological Analysis Method. Environmental Product Declaration. 3M.
- Huang, J., R. B. Kogbara, N. Hariharan, E. A. Masad, and D. N. Little. 2021a. "A state-of-the-art review of polymers used in soil stabilization." *Construction and Building Materials*, 305: 124685. <https://doi.org/10.1016/j.conbuildmat.2021.124685>.

- Huang, J., R. B. Kogbara, N. Hariharan, E. A. Masad, and D. N. Little. 2021b. "A state-of-the-art review of polymers used in soil stabilization." *Construction and Building Materials*, 305: 124685. <https://doi.org/10.1016/j.conbuildmat.2021.124685>.
- Huang, S.-C., and M. Zeng. 2007. "Characterization of aging effect on rheological properties of asphalt-filler systems." *International Journal of Pavement Engineering*, 8 (3): 213–223. Taylor & Francis. <https://doi.org/10.1080/10298430601135477>.
- "Investigating the rheological properties of asphalt binder incorporating different crumb rubber contents based on a response surface methodology | Elsevier Enhanced Reader." n.d. Accessed January 17, 2023. <https://reader.elsevier.com/reader/sd/pii/S1018363920303305?token=F1AE346167CF4F62F7976B32B98CEE6D4C790DAE92FE55F82779C665BCF1CB2BDC55DC427763892AB88A3597456B7CEF&originRegion=us-east-1&originCreation=20230118003826>.
- Islam, M. R., and R. A. Tarefder. 2020. *Pavement design: materials, analysis, and highways*. New York: McGraw Hill.
- Islam, M. S., K. L. Pickering, and N. J. Foreman. 2010. "Influence of Hygrothermal Ageing on the Physico-Mechanical Properties of Alkali Treated Industrial Hemp Fibre Reinforced Polylactic Acid Composites." *J Polym Environ*, 18 (4): 696–704. <https://doi.org/10.1007/s10924-010-0225-9>.
- Islam, M. Z., M. E. Sarker, M. M. Rahman, M. R. Islam, A. T. M. F. Ahmed, M. S. Mahmud, and M. Syduzzaman. 2022. "Green composites from natural fibers and biopolymers: A review on processing, properties, and applications." *Journal of Reinforced Plastics and Composites*, 41 (13–14): 526–557. <https://doi.org/10.1177/07316844211058708>.
- Jagan Gudimettla, A. Cooley Jr, and E. Ray Brown. 2004. "Workability of hot mix asphalt." *Transportation Research Record: Journal of the Transportation Research Board*, 1891: 229–237. <https://doi.org/10.3141/1891-27>.
- Jalilian, N., G. D. Najafpour, and M. Khajouei. 2020. "Macro and Micro Algae in Pollution Control and Biofuel Production – A Review." *ChemBioEng Reviews*, 7 (1): 18–33. <https://doi.org/10.1002/cben.201900014>.
- Jang, J. 2020. "A Review of the Application of Biopolymers on Geotechnical Engineering and the Strengthening Mechanisms between Typical Biopolymers and Soils." *Advances in Materials Science and Engineering*, 2020: e1465709. Hindawi. <https://doi.org/10.1155/2020/1465709>.
- Jiang, T., J. Zhao, and J. Zhang. 2022. "Splitting tensile strength and microstructure of xanthan gum-treated loess." *Sci Rep*, 12 (1): 9921. Nature Publishing Group. <https://doi.org/10.1038/s41598-022-14058-4>.
- Joana Peralta, Hugo M. R. D. Silva, R. Christopher Williams, Marjorie Rover, and Ana V. A. Machado. 2013. "Development of an Innovative Bio-Binder Using Asphalt-Rubber

- Technology.” *International Journal of Pavement Research and Technology*, 6 (4).
[https://doi.org/10.6135/ijprt.org.tw/2013.6\(4\).447](https://doi.org/10.6135/ijprt.org.tw/2013.6(4).447).
- Jones, A., M. A. Zeller, and S. Sharma. 2013. “Thermal, mechanical, and moisture absorption properties of egg white protein bioplastics with natural rubber and glycerol.” *Prog Biomater*, 2 (1): 12. <https://doi.org/10.1186/2194-0517-2-12>.
- Kantesaria, N., P. Chandra, and A. Sachan. n.d. “Stabilization of Expansive Soil Using Agar Biopolymer.” Accessed May 15, 2023.
<https://ascelibrary.org/doi/epdf/10.1061/9780784483411.026>.
- Kaseer, F., A. E. Martin, and E. Arámbula-Mercado. 2019. “Use of recycling agents in asphalt mixtures with high recycled materials contents in the United States: A literature review.” *Construction and Building Materials*, 211: 974–987.
<https://doi.org/10.1016/j.conbuildmat.2019.03.286>.
- Khatami, H. R., and B. C. O’Kelly. 2013. “Improving Mechanical Properties of Sand Using Biopolymers.” *Journal of Geotechnical and Geoenvironmental Engineering*, 139 (8): 1402–1406. American Society of Civil Engineers.
[https://doi.org/10.1061/\(ASCE\)GT.1943-5606.0000861](https://doi.org/10.1061/(ASCE)GT.1943-5606.0000861).
- Kheradmand, B., R. Muniandy, L. T. Hua, R. Bt. Yunus, and A. Solouki. 2014. “An overview of the emerging warm mix asphalt technology.” *International Journal of Pavement Engineering*, 15 (1): 79–94. Taylor & Francis.
<https://doi.org/10.1080/10298436.2013.839791>.
- Kim, B., K. La Flamme, and N. A. Peppas. 2003. “Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery.” *Journal of Applied Polymer Science*, 89 (6): 1606–1613. <https://doi.org/10.1002/app.12337>.
- Koenig, A. 2020. “Analysis of air voids in cementitious materials using micro X-ray computed tomography (μ XCT).” *Construction and Building Materials*, 244: 118313.
<https://doi.org/10.1016/j.conbuildmat.2020.118313>.
- Kraan, S. 2012. “Algal Polysaccharides, Novel Applications and Outlook.” *Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology*, C.-F. Chang, ed. InTech.
- Kusmono, and I. Abdurrahim. 2019. “Water sorption, antimicrobial activity, and thermal and mechanical properties of chitosan/clay/glycerol nanocomposite films.” *Heliyon*, 5 (8): e02342. <https://doi.org/10.1016/j.heliyon.2019.e02342>.
- Labropoulos, K. C., S. Rangarajan, D. E. Niesz, and S. C. Danforth. 2001. “Dynamic Rheology of Agar Gel Based Aqueous Binders.” *Journal of the American Ceramic Society*, 84 (6): 1217–1224. <https://doi.org/10.1111/j.1151-2916.2001.tb00819.x>.
- Laurens, L. M. L., M. Lane, and R. S. Nelson. 2020. “Sustainable seaweed biotechnology solutions for carbon capture, composition, and deconstruction.” *Trends in Biotechnology*, 38 (11). <https://doi.org/10.1016/j.tibtech.2020.03.015>.

- Lavorgna, M., F. Piscitelli, P. Mangiacapra, and G. G. Buonocore. 2010. "Study of the combined effect of both clay and glycerol plasticizer on the properties of chitosan films." *Carbohydrate Polymers*, 82 (2): 291–298. <https://doi.org/10.1016/j.carbpol.2010.04.054>.
- Lee, W.-K., Y.-Y. Lim, A. T.-C. Leow, P. Namasivayam, J. O. Abdullah, and C. L. Ho. 2017. "Biosynthesis of agar in red seaweeds: A review." *Carbohydrate Polymers*, 164: 23–30. <https://doi.org/10.1016/j.carbpol.2017.01.07>.
- Li, J., F. Zhang, Y. Muhammad, Y. Liu, Y. Wei, and H. Chen. 2019. "Fabrication and properties of wide temperature domain pavement seaweed modified bio-bitumen." *Construction and Building Materials*, 227: 117079. <https://doi.org/10.1016/j.conbuildmat.2019.117079>.
- Linden, R., J. Mahoney, and N. Jackson. 1989. "Effect of compaction on asphalt concrete performance." *Transportation Research Record*, 1217: 20–28.
- Little, D. N., D. H. Allen, and A. Bhasin. 2018. *Modeling and Design of Flexible Pavements and Materials*. Cham: Springer International Publishing.
- Liu, Y., X. Liu, B. Duan, Z. Yu, T. Cheng, L. Yu, L. Liu, and K. Liu. 2021. "Polymer–Water Interaction Enabled Intelligent Moisture Regulation in Hydrogels." *J. Phys. Chem. Lett.*, 12 (10): 2587–2592. <https://doi.org/10.1021/acs.jpclett.1c00034>.
- Long Jiang, E. Morelius, Jinwen Zhang, M. Wolcott, and J. Holbery. 2008. "Study of the Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/Cellulose Nanowhisker Composites Prepared by Solution Casting and Melt Processing." *Journal of Composite Materials*, 42 (24): 2629–2645. <https://doi.org/10.1177/0021998308096327>.
- Losini, A. E., A. C. Grillet, M. Bellotto, M. Woloszyn, and G. Dotelli. 2021. "Natural additives and biopolymers for raw earth construction stabilization – a review." *Construction and Building Materials*, 304: 124507. <https://doi.org/10.1016/j.conbuildmat.2021.124507>.
- Lv, S., C. Xia, Q. Yang, S. Guo, L. You, Y. Guo, and J. Zheng. 2020. "Improvements on high-temperature stability, rheology, and stiffness of asphalt binder modified with waste crayfish shell powder." *Journal of Cleaner Production*, 264: 121745. <https://doi.org/10.1016/j.jclepro.2020.121745>.
- Lyons, J. G., L. M. Geever, M. J. D. Nugent, J. E. Kennedy, and C. L. Higginbotham. 2009. "Development and characterisation of an agar–polyvinyl alcohol blend hydrogel." *Journal of the Mechanical Behavior of Biomedical Materials*, 2 (5): 485–493. <https://doi.org/10.1016/j.jmbbm.2008.12.003>.
- Madera-Santana, T. J., D. Robledo, A. Azamar, and C. R. Rios-Soberanis. 2010. "Preparation and characterization of low density polyethylene-agar biocomposites: Torque-rheological, mechanical, thermal and morphological properties." *Polymer Engineering and Science*, 50 (3): 585–591. <https://doi.org/10.1002/pen.21574>.
- Magalhães, S., L. Alves, B. Medronho, A. C. Fonseca, A. Romano, J. F. J. Coelho, and M. Norgren. 2019. "Brief Overview on Bio-Based Adhesives and Sealants." *Polymers*, 11

- (10): 1685. Multidisciplinary Digital Publishing Institute.
<https://doi.org/10.3390/polym11101685>.
- Malabi Eberhardt, L. C., J. Rønholt, M. Birkved, and H. Birgisdottir. 2021. "Circular Economy potential within the building stock - Mapping the embodied greenhouse gas emissions of four Danish examples." *Journal of Building Engineering*, 33: 101845.
<https://doi.org/10.1016/j.jobe.2020.101845>.
- Mallick, R. B., and T. El-Korchi. 2018a. *Pavement engineering: principles and practice*. Boca Raton: CRC Press/Taylor & Francis Group.
- Mallick, R. B., and T. El-Korchi. 2018b. "Asphalt and Emulsions." *Pavement engineering: principles and practice*. Boca Raton: CRC Press/Taylor & Francis Group.
- Mao, B., A. Bentaleb, F. Louerat, T. Divoux, and P. Snabre. 2017. "Heat-induced aging of agar solutions: Impact on the structural and mechanical properties of agar gels." *Food Hydrocolloids*, 64: 59–69. <https://doi.org/10.1016/j.foodhyd.2016.10.020>.
- Miandad, R., M. Rehan, M. A. Barakat, A. S. Aburizaiza, H. Khan, I. M. I. Ismail, J. Dhavamani, J. Gardy, A. Hassanpour, and A.-S. Nizami. 2019. "Catalytic Pyrolysis of Plastic Waste: Moving Toward Pyrolysis Based Biorefineries." *Front. Energy Res.*, 7: 27. <https://doi.org/10.3389/fenrg.2019.00027>.
- Mohan, H. T., K. Jayanarayanan, and K. M. Mini. 2021. "Recent trends in utilization of plastics waste composites as construction materials." *Construction and Building Materials*, 271: 121520. <https://doi.org/10.1016/j.conbuildmat.2020.121520>.
- Moretti, L., P. Di Mascio, and C. Fusco. 2019. "Porous Concrete for Pedestrian Pavements." *Water*, 11 (10): 2105. <https://doi.org/10.3390/w11102105>.
- Mori, R. 2023. "Replacing all petroleum-based chemical products with natural biomass-based chemical products: a tutorial review." *RSC Sustain.*, 1 (2): 179–212. <https://doi.org/10.1039/D2SU00014H>.
- Mostafavi, F. S., and D. Zaeim. 2020. "Agar-based edible films for food packaging applications - A review." *International Journal of Biological Macromolecules*, 159: 1165–1176. <https://doi.org/10.1016/j.ijbiomac.2020.05.123>.
- Muguda, S., S. J. Booth, P. N. Hughes, C. E. Augarde, C. Perlot, A. W. Bruno, and D. Gallipoli. 2017. "Mechanical properties of biopolymer-stabilised soil-based construction materials." *Géotechnique Letters*, 7 (4): 309–314. ICE Publishing. <https://doi.org/10.1680/jgele.17.00081>.
- Mugume, R., and D. Kakoto. 2020. "Effect of inappropriate binder grade selection on initiation of asphalt pavement cracking." *Sustainability*, 12 (6099): 1–18. <https://doi.org/10.3390/su12156099>.

- Nanda, S., B. R. Patra, R. Patel, J. Bakos, and A. K. Dalai. 2022. "Innovations in applications and prospects of bioplastics and biopolymers: a review." *Environ Chem Lett*, 20 (1): 379–395. <https://doi.org/10.1007/s10311-021-01334-4>.
- Nasef, M., E. Elhefian, S. Saalah, and A. Yahaya. 2011. "Preparation and Properties of Non-Crosslinked and Ionically Crosslinked Chitosan/Agar Blended Hydrogel Films." *Journal of Chemistry*, 8. <https://doi.org/10.1155/2011/513204>.
- National Institute of Standards and Technology. n.d. "NIST Chemistry WebBook, SRD 69-Calcium Carbonate (precipitated) IR Spectra."
- Naveed, H., Z. ur Rehman, A. Hassan Khan, S. Qamar, and M. N. Akhtar. 2019. "Effect of mineral fillers on the performance, rheological and dynamic viscosity measurements of asphalt mastic." *Construction and Building Materials*, 222: 390–399. <https://doi.org/10.1016/j.conbuildmat.2019.06.170>.
- Nishinari, K., and Y. Fang. 2017. "Relation between structure and rheological/thermal properties of agar. A mini review on the effect of alkali treatment and the role of agaropectin." *Food Structure*, 13: 24–34. <https://doi.org/10.1016/j.foostr.2016.10.003>.
- Nissa, R. C., A. K. Fikriyyah, A. H. D. Abdullah, and S. Pudjiraharti. 2019. "Preliminary study of biodegradability of starch-based bioplastics using ASTM G21-70, dip-hanging, and Soil Burial Test methods." *IOP Conf. Ser.: Earth Environ. Sci.*, 277: 012007. <https://doi.org/10.1088/1755-1315/277/1/012007>.
- Norziah, M. H., S. L. Foo, and A. Abd. Karim. 2006. "Rheological studies on mixtures of agar (*Gracilaria changii*) and κ -carrageenan." *Food Hydrocolloids*, 7th International Hydrocolloids Conference, 20 (2): 204–217. <https://doi.org/10.1016/j.foodhyd.2005.03.020>.
- Nwankwo, C. O., J. Mahachi, D. O. Olukanni, and I. Musonda. 2023. "Natural fibres and biopolymers in FRP composites for strengthening concrete structures: A mixed review." *Construction and Building Materials*, 363: 129661. <https://doi.org/10.1016/j.conbuildmat.2022.129661>.
- Obahiagbon, K., A. Amenaghawon, and C. Onyia. 2014. "Prevention of Biodeterioration of Crude Oil in Tanks Using Anti-Microbial Agents." *International Journal of Scientific Research in Environmental Sciences*, 2: 56–62. <https://doi.org/10.12983/ijres-2014-p0056-0062>.
- Oberti, I., and A. Paciello. 2022. "Bioplastic as a Substitute for Plastic in Construction Industry." *Encyclopedia*, 2 (3): 1408–1420. <https://doi.org/10.3390/encyclopedia2030095>.
- Object Research Systems. 2019. *DragonFly Release Notes*. 1–68.
- Özçimen, D., B. İnan, O. morkoç, and A. Efe. 2017. "A Review on Algal Biopolymers." *Journal of Chemical Engineering Research Updates*, 4: 7–14. <https://doi.org/10.15377/2409-983X.2017.04.2>.

- Pahlavan, F., D. Oldham, S. Shakiba, S. Louie, and E. Fini. 2021. "Protein enriched biowaste: A viable feedstock to make durable bio-binders for bituminous composites." *Resources, Conservation and Recycling*, 170: 105576. <https://doi.org/10.1016/j.resconrec.2021.105576>.
- Pahlavan, F., A. Rajib, S. Deng, P. Lammers, and E. H. Fini. 2020. "Investigation of Balanced Feedstocks of Lipids and Proteins To Synthesize Highly Effective Rejuvenators for Oxidized Asphalt." *ACS Sustainable Chem. Eng.*, 8 (20): 7656–7667. <https://doi.org/10.1021/acssuschemeng.0c01100>.
- Park, K.-B., J.-S. Kim, F. Pahlavan, and E. H. Fini. 2022. "Biomass Waste to Produce Phenolic Compounds as Antiaging Additives for Asphalt." *ACS Sustainable Chem. Eng.*, 10 (12): 3892–3908. <https://doi.org/10.1021/acssuschemeng.1c07870>.
- Parsons, S., M. J. Allen, F. Abeln, M. McManus, and C. J. Chuck. 2019. "Sustainability and life cycle assessment (LCA) of macroalgae-derived single cell oils." *Journal of Cleaner Production*, 232: 1272–1281. <https://doi.org/10.1016/j.jclepro.2019.05.315>.
- Paul, V., P. S. Chandra Shekharaiah, S. Kushwaha, A. Sapre, S. Dasgupta, and D. Sanyal. 2020. "Role of Algae in CO₂ Sequestration Addressing Climate Change: A Review." *Renewable Energy and Climate Change, Smart Innovation, Systems and Technologies*, D. Deb, A. Dixit, and L. Chandra, eds., 257–265. Singapore: Springer.
- Penki, R., and S. K. Rout. 2021. "Next-generation bitumen: a review on challenges and recent developments in bio-bitumen preparation and usage." *Biomass Conv. Bioref.* <https://doi.org/10.1007/s13399-021-01803-4>.
- Pereira, L., A. Sousa, H. Coelho, A. M. Amado, and P. J. A. Ribeiro-Claro. 2003. "Use of FTIR, FT-Raman and ¹³C-NMR spectroscopy for identification of some seaweed phycocolloids." *Biomolecular Engineering, Marine Biotechnology: Basics and Applications*, 20 (4): 223–228. [https://doi.org/10.1016/S1389-0344\(03\)00058-3](https://doi.org/10.1016/S1389-0344(03)00058-3).
- Plank, J. 2004. "Applications of biopolymers and other biotechnological products in building materials." *Appl Microbiol Biotechnol*, 66 (1): 1–9. <https://doi.org/10.1007/s00253-004-1714-3>.
- du Plessis, A., and W. P. Boshoff. 2019. "A review of X-ray computed tomography of concrete and asphalt construction materials." *Construction and Building Materials*, 199: 637–651. <https://doi.org/10.1016/j.conbuildmat.2018.12.049>.
- Portugal, A. C. X., L. C. de F. Lopes Lucena, A. E. de F. Lopes Lucena, D. B. Costa, and K. A. de Lima. 2017. "Rheological Properties of Asphalt Binders Prepared with Maize Oil." *Construction and Building Materials*, 152: 1015–1026. <https://doi.org/10.1016/j.conbuildmat.2017.07.077>.
- Pourjavadi, A., B. Farhadpour, and F. Seidi. 2009. "Synthesis and investigation of swelling behavior of new agar based superabsorbent hydrogel as a candidate for agrochemical delivery." *J Polym Res*, 16 (6): 655–665. <https://doi.org/10.1007/s10965-009-9270-2>.

- Powrie, W. 2014. "Origin and classification of soils." *Soil Mechanics*. CRC Press.
- Rabbat, C., S. Awad, A. Villot, D. Rollet, and Y. Andr  s. 2022. "Sustainability of biomass-based insulation materials in buildings: Current status in France, end-of-life projections and energy recovery potentials." *Renewable and Sustainable Energy Reviews*, 156: 111962. <https://doi.org/10.1016/j.rser.2021.111962>.
- Ragab, A. A., R. K. Farag, U. F. Kandil, M. El-Shafie, A. M. M. Saleh, and A. F. El-Kafrawy. 2016. "Thermo-mechanical properties improvement of asphalt binder by using methylmethacrylate/ethylene glycol dimethacrylate." *Egyptian Journal of Petroleum*, 25 (3): 397–407. <https://doi.org/10.1016/j.ejpe.2015.09.001>.
- Rani, G. U., A. K. Konreddy, and S. Mishra. 2018. "Novel hybrid biosorbents of agar: Swelling behaviour, heavy metal ions and dye removal efficacies." *International Journal of Biological Macromolecules*, 117: 902–910. <https://doi.org/10.1016/j.ijbiomac.2018.05.163>.
- Raouf, M. A., and C. R. Williams. 2010a. "Temperature and Shear Susceptibility of a Nonpetroleum Binder as a Pavement Material." *Transportation Research Record: Journal of the Transportation Research Board*, 2180: 9–18. <https://doi.org/10.3141/2180-02>.
- Raouf, M. A., and C. R. Williams. 2010b. "General rheological properties of fractionated switchgrass bio-oil as a pavement material." *Road Materials and Pavement Design*, 11: 325–353. <https://doi.org/10.1080/14680629.2010.9690337>.
- Reclaimed Asphalt Pavement in Asphalt Mixtures: State of the Practice. 2011. Federal Highway Administration.
- Reghunadhan, A., and S. Thomas. 2017. "Chapter 1 - Polyurethanes: Structure, Properties, Synthesis, Characterization, and Applications." *Polyurethane Polymers*, S. Thomas, J. Datta, J. T. Haponiuk, and A. Reghunadhan, eds., 1–16. Amsterdam: Elsevier.
- Robinette, C., and J. Epps. 2010. "Energy, Emissions, Material Conservation, and Prices Associated with Construction, Rehabilitation, and Material Alternatives for Flexible Pavement." *Transportation Research Record*, 2179 (1): 10–22. <https://doi.org/10.3141/2179-02>.
- Rubio, M. C., G. Martinez, L. Baena, and F. Moreno. 2012. "Warm mix asphalt: an overview." *Journal of Cleaner Production*, 24: 76–84.
- Rubio, M. del C., F. Moreno, M. J. Mart  nez-Echevarr  a, G. Mart  nez, and J. M. V  zquez. 2013. "Comparative analysis of emissions from the manufacture and use of hot and half-warm mix asphalt." *Journal of Cleaner Production*, 41: 1–6. <https://doi.org/10.1016/j.jclepro.2012.09.036>.
- S   Araujo, M. F. A., L. F. M. Leite, V. M. D. Pasa, and V. F. C. Lins. 2014. "RHEOLOGICAL AND THERMAL BEHAVIOR OF WEATHERING-AGED POLYMER MODIFIED BITUMEN." *BJPG*, 7 (4): 155–167. <https://doi.org/10.5419/bjpg2013-0013>.

- Sahoo, D., G. Elangbam, and S. devi. 2012. "Using algae for carbon dioxide capture and bio-fuel production to combat climate change." *Phykos*, 42(1).
- Sahoo, D., and J. Seckbach (Eds.). 2015. *The Algae World. Cellular Origin, Life in Extreme Habitats and Astrobiology*. Dordrecht: Springer Netherlands.
- Sajid, M. A., S. A. Shahzad, F. Hussain, W. G. Skene, Z. A. Khan, and M. Yar. 2018. "Synthetic modifications of chitin and chitosan as multipurpose biopolymers: A review." *Synthetic Communications*, 48 (15): 1893–1908. Taylor & Francis.
<https://doi.org/10.1080/00397911.2018.1465096>.
- Salvo, L., M. Suéry, A. Marmottant, N. Limodin, and D. Bernard. 2010. "3D imaging in material science: Application of X-ray tomography." *Comptes Rendus Physique, Interactions between radiofrequency signals and living organisms*, 11 (9): 641–649.
<https://doi.org/10.1016/j.crhy.2010.12.003>.
- Santos, V. H. J. M. dos, D. Pontin, G. G. D. Ponzi, A. S. de G. e Stepanha, R. B. Martel, M. K. Schütz, S. M. O. Einloft, and F. Dalla Vecchia. 2021. "Application of Fourier Transform infrared spectroscopy (FTIR) coupled with multivariate regression for calcium carbonate (CaCO₃) quantification in cement." *Construction and Building Materials*, 313: 125413.
<https://doi.org/10.1016/j.conbuildmat.2021.125413>.
- Sastri, V. R. 2010. "Chapter 7 - Engineering Thermoplastics: Acrylics, Polycarbonates, Polyurethanes, Polyacetals, Polyesters, and Polyamides." *Plastics in Medical Devices, Plastics Design Library*, V. R. Sastri, ed., 121–173. Boston: William Andrew Publishing.
- Semachai, T., P. Chandranupap, and P. Chandranupap. 2018. "Preparation of Microcrystalline Cellulose from Water Hyacinth Reinforced Polylactic Acid Biocomposite." *MATEC Web Conf.*, (J. Wang and Y. F. Yang, eds.), 187: 02003.
<https://doi.org/10.1051/mateconf/201818702003>.
- Shalbafan, A., and H. Thoemen. 2020. "Geopolymer-Bonded Laminated Veneer Lumber as Environmentally Friendly and Formaldehyde-Free Product: Effect of Various Additives on Geopolymer Binder Features." *Applied Sciences*, 10 (2): 593. Multidisciplinary Digital Publishing Institute. <https://doi.org/10.3390/app10020593>.
- Singh, R., S. Gautam, B. Sharma, P. Jain, and K. D. Chauhan. 2021. "Chapter 2 - Biopolymers and their classifications." *Biopolymers and their Industrial Applications*, S. Thomas, S. Gopi, and A. Amalraj, eds., 21–44. Elsevier.
- Sionkowska, A. 2014. "The Processes Induced by UV Light in Biopolymers and Biopolymer Composites." *Molecular Crystals and Liquid Crystals*, 590 (1): 17–23.
<https://doi.org/10.1080/15421406.2013.873624>.
- Siracusa, V., and I. Blanco. 2020. "Bio-Polyethylene (Bio-PE), Bio-Polypropylene (Bio-PP) and Bio-Poly(ethylene terephthalate) (Bio-PET): Recent Developments in Bio-Based Polymers Analogous to Petroleum-Derived Ones for Packaging and Engineering

- Applications.” *Polymers*, 12 (8): 1641. Multidisciplinary Digital Publishing Institute. <https://doi.org/10.3390/polym12081641>.
- Smitha, S., K. Rangaswamy, and D. S. Keerthi. 2021. “Triaxial test behaviour of silty sands treated with agar biopolymer.” *International Journal of Geotechnical Engineering*, 15 (4): 484–495. <https://doi.org/10.1080/19386362.2019.1679441>.
- Smitha, S., and A. Sachan. 2016. “Use of agar biopolymer to improve the shear strength behavior of sabarmati sand.” *International Journal of Geotechnical Engineering*, 10 (4): 387–400. Taylor & Francis. <https://doi.org/10.1080/19386362.2016.1152674>.
- Soldo, A., M. Miletić, and M. L. Auad. 2020. “Biopolymers as a sustainable solution for the enhancement of soil mechanical properties.” *Sci Rep*, 10 (1): 267. Nature Publishing Group. <https://doi.org/10.1038/s41598-019-57135-x>.
- Somboon, N., T. T. Karrila, T. Kaewmanee, and S. J. Karrila. 2014. “Properties of gels from mixed agar and fish gelatin.” *International Food Research Journal*, 21 (2): 485–492.
- Southern, M. 2015. “A perspective of bituminous binder specifications.” *Advances in Asphalt Materials*, 1–27. Elsevier.
- Speight, J. G. 2016. “Asphalt Materials Science and Technology”, Elsevier. Boston: Butterworth-Heinemann.
- Srubar, W. V., S. Pilla, Z. C. Wright, C. A. Ryan, J. P. Greene, C. W. Frank, and S. L. Billington. 2012. “Mechanisms and impact of fiber–matrix compatibilization techniques on the material characterization of PHBV/oak wood flour engineered biobased composites.” *Composites Science and Technology*, 72 (6): 708–715. <https://doi.org/10.1016/j.compscitech.2012.01.021>.
- Stefanowski, B. K., S. F. Curling, and G. A. Ormondroyd. 2017. “A rapid screening method to determine the susceptibility of bio-based construction and insulation products to mould growth.” *International Biodeterioration & Biodegradation*, 116: 124–132. <https://doi.org/10.1016/j.ibiod.2016.10.025>.
- Sukhija, M., and N. Saboo. 2021. “A comprehensive review of warm mix asphalt mixtures-laboratory to field.” *Construction and Building Materials*, 274: 121781. <https://doi.org/10.1016/j.conbuildmat.2020.121781>.
- Sukumaran, N. P., and S. Gopi. 2021. “Chapter 1 - Overview of biopolymers: resources, demands, sustainability, and life cycle assessment modeling and simulation.” *Biopolymers and their Industrial Applications*, S. Thomas, S. Gopi, and A. Amalraj, eds., 1–19. Elsevier.
- Sun, J., O. Norouzi, and O. Mašek. 2022. “A state-of-the-art review on algae pyrolysis for bioenergy and biochar production.” *Bioresource Technology*, 346: 126258. <https://doi.org/10.1016/j.biortech.2021.126258>.

- Taelman, S. E., J. Champenois, M. D. Edwards, S. De Meester, and J. Dewulf. 2015. "Comparative environmental life cycle assessment of two seaweed cultivation systems in North West Europe with a focus on quantifying sea surface occupation." *Algal Research*, 11: 173–183. <https://doi.org/10.1016/j.algal.2015.06.018>.
- Tanase, E. E., M. Rapa, and O. Popa. 2014. "Biopolymers Based on Renewable Resources-A review." *Scientific Bulletin, Series F, Biotechnologies Vol XVIII*.
- Tarsi, G., P. Tataranni, and C. Sangiorgi. 2020. "The Challenges of Using Reclaimed Asphalt Pavement for New Asphalt Mixtures: A Review." *Materials*, 13 (18): 4052. <https://doi.org/10.3390/ma13184052>.
- Thives, L. P., and E. Ghisi. 2017. "Asphalt Mixtures Emission and Energy Consumption: A review." *Renewable and Sustainable Energy Reviews*, 72: 473–484. <https://doi.org/10.1016/j.rser.2017.01.087>.
- Thom, N. 2014. *Principles of Pavement Engineering*. Westminster, London: ICE Publishing.
- Utech, S., and A. R. Boccaccini. 2016. "A review of hydrogel-based composites for biomedical applications: enhancement of hydrogel properties by addition of rigid inorganic fillers." *J Mater Sci*, 51 (1): 271–310. <https://doi.org/10.1007/s10853-015-9382-5>.
- Verma, A. K., A. Prasad, and N. S. Bonal. 2023. "Investigation of the long-term shear strength behavior of municipal solid waste fines stabilized with biopolymer: An experimental study." *Journal of Environmental Chemical Engineering*, 11 (3): 109805. <https://doi.org/10.1016/j.jece.2023.109805>.
- Vijay Anand, K. G., K. Eswaran, and A. Ghosh. 2018. "Life cycle impact assessment of a seaweed product obtained from *Gracilaria edulis* – A potent plant biostimulant." *Journal of Cleaner Production*, 170: 1621–1627. <https://doi.org/10.1016/j.jclepro.2017.09.241>.
- Vinod, A., M. R. Sanjay, S. Suchart, and P. Jyotishkumar. 2020. "Renewable and sustainable biobased materials: An assessment on biofibers, biofilms, biopolymers and biocomposites." *Journal of Cleaner Production*, 258: 120978. <https://doi.org/10.1016/j.jclepro.2020.120978>.
- Vishweshwaran, M., and E. R. Sujatha. 2023. "Geotechnical Investigation of Gelatin Biopolymer on Cohesive Soils." *Sustainability*, 15 (3): 2041. <https://doi.org/10.3390/su15032041>.
- Vuai, S. A. H. 2022. "Characterization of agar extracted from *Gracilaria* species collected along Tanzanian coast." *Heliyon*, 8 (2): e09002. <https://doi.org/10.1016/j.heliyon.2022.e09002>.
- Wang, S.-M., Q.-Z. Huang, and Q.-S. Wang. 2005. "Study on the synergetic degradation of chitosan with ultraviolet light and hydrogen peroxide." *Carbohydrate Research*, 340 (6): 1143–1147. <https://doi.org/10.1016/j.carres.2005.02.009>.

- Wei, X.-F., E. Linde, and M. S. Hedenqvist. 2019. "Plasticiser loss from plastic or rubber products through diffusion and evaporation." *npj Mater Degrad*, 3 (1): 18. <https://doi.org/10.1038/s41529-019-0080-7>.
- Williams, E. G. 1961. "When to use what grade of asphalt and why." *Materials Science*, 218–228.
- Williams, S., D. Beatty, and W. V. Srubar. n.d. "Towards Living Construction Materials: Progress and Potential."
- Xu, X., S. Xu, L. Jin, and E. Song. 2011. "Characteristic analysis of Otsu threshold and its applications." *Pattern Recognition Letters*, 32 (7): 956–961. <https://doi.org/10.1016/j.patrec.2011.01.021>.
- Yamane, K., H. Sato, Y. Ichikawa, K. Sunagawa, and Y. Shigaki. 2014. "Development of an industrial production technology for high-molecular-weight polyglycolic acid." *Polym J*, 46 (11): 769–775. <https://doi.org/10.1038/pj.2014.69>.
- Yan, S., C. Zhou, and Y. Sun. 2022. "Evaluation of Rejuvenated Aged-Asphalt Binder by Waste-Cooking Oil with Secondary Aging Considered." *Journal of Materials in Civil Engineering*, 34 (8): 04022157. American Society of Civil Engineers. [https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0004306](https://doi.org/10.1061/(ASCE)MT.1943-5533.0004306).
- Yang, C., X. Xing, Z. Li, and S. Zhang. 2020. "A Comprehensive Review on Water Diffusion in Polymers Focusing on the Polymer–Metal Interface Combination." *Polymers*, 12 (1): 138. <https://doi.org/10.3390/polym12010138>.
- Yang, L., and A. T. Paulson. 2000. "Mechanical and water vapour barrier properties of edible gellan films." *Food Research International*, 33 (7): 563–570. [https://doi.org/10.1016/S0963-9969\(00\)00092-2](https://doi.org/10.1016/S0963-9969(00)00092-2).
- Ye, S., Y. Zhang, J. Chen, F. Chen, H. Weng, Q. Xiao, and A. Xiao. 2022a. "Synthesis and properties of maleic anhydride-modified agar with reversibly controlled gel strength." *International Journal of Biological Macromolecules*, 201: 364–377. <https://doi.org/10.1016/j.ijbiomac.2021.12.096>.
- Yemashova, N. A., V. P. Murygina, D. V. Zhukov, A. A. Zakharyantz, M. A. Gladchenko, V. Appanna, and S. V. Kalyuzhnyi. 2007. "Biodeterioration of crude oil and oil derived products: a review." *Rev Environ Sci Biotechnol*, 6 (4): 315–337. <https://doi.org/10.1007/s11157-006-9118-8>.
- Zahoor, M., S. Nizamuddin, S. Madapusi, and F. Giustozzi. 2021. "Sustainable asphalt rejuvenation using waste cooking oil: A comprehensive review." *Journal of Cleaner Production*, 278: 123304. <https://doi.org/10.1016/j.jclepro.2020.123304>.
- Zhang, L., C. Xing, F. Gao, T. Li, and Y. Tan. 2016. "Using DSR and MSCR tests to characterize high temperature performance of different rubber modified asphalt."

- Construction and Building Materials, 127: 466–474.
<https://doi.org/10.1016/j.conbuildmat.2016.10.010>.
- Zhang, X., K. Zhang, C. Wu, K. Liu, and K. Jiang. 2020. “Preparation of bio-oil and its application in asphalt modification and rejuvenation: A review of the properties, practical application and life cycle assessment.” *Construction and Building Materials*, 262: 120528. <https://doi.org/10.1016/j.conbuildmat.2020.120528>.
- Zhang, Z., Y. Fang, J. Yang, and X. Li. 2022. “A comprehensive review of bio-oil, bio-binder and bio-asphalt materials: Their source, composition, preparation and performance.” *Journal of Traffic and Transportation Engineering (English Edition)*, S2095756422000228. <https://doi.org/10.1016/j.jtte.2022.01.003>.
- Zhenhua, Y., Z. Jianbo, W. Hao, Z. Han, X. Jiao, and W. Xu. 2020. “Analysis of influencing factors on viscosity of agar solution for capsules.” *Journal of Physics: Conference Series*, 1653. <https://doi.org/10.1088/1742-6596/1653/1/012059>.
- Zia, K. M., M. Zuber, and M. Ali. 2017. *Algae Based Polymers, Blends, and Composites: Chemistry, Biotechnology and Materials Science*. Elsevier.