EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF MODIFIED ALGAE PROTEIN ADHESIVES

by

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Abstract

Algae proteins have similar amino acid compositions as conventional plant proteins, and are comparatively richer in the essential amino acids. Algae protein has the potential to be used in the development of a wide variety of products, including foods, animal feeds, bioplastics, and adhesives. The utilization of algae protein for value-added products would increase the economic feasibility of algae biodiesel. This research evaluated the adhesion, rheological, morphological, and thermal properties of adhesives made from algae protein extracted from *Cladophora* sp. and modified with either sodium hydroxide (pH 9, 10, 11) or sodium dodecyl sulfate (SDS, 0.5, 1, and 3%).

Both alkali-modified and SDS-modified algae protein adhesives displayed improved dry shear strength compared to unmodified algae protein. However, only 3% SDS-modified algae protein significantly improved the water resistance as shown in wet and soak shear strength tests. Thermal analysis using differential scanning calorimetry showed that SDS modification caused complete denaturation of the algae protein. SDS modification also increased the viscosity of the adhesive and created rougher particle surface texture. These data suggest that SDS modification can effectively increase shear strength and water resistance of algae protein adhesives caused by protein denaturation and protein structure change.

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Chapter 1 - Introduction

Due to the inherently finite nature of fossil fuel resources, the world faces the challenge of finding suitable renewable substitutes that can begin to replace petrochemicals both as a source of energy and as a source of materials for plastics, rubbers, fertilizers, and fine chemicals. One of the possible alternatives to petroleum-based fuels and products is biomass such as algae (Chisti 2007). Algae biomass contains lipids, proteins, and carbohydrates that can be processed into fuels or other valuable co-products through chemical, biochemical, or thermochemical means (Sheehan et al. 1998). The lipids are of particular interest in current research due to the ability to use the algal oils to produce biodiesel. Algae stands out from other sources of biomass with respect to lipid production with some estimates stating that algae is capable of producing up to 30 times as much oil per unit area of land as conventional oilseed crops under ideal conditions (Sheehan et al. 1998). Additionally, algae has the added benefit of not competing with traditional food crops because it can be grown on marginal lands and can utilize brackish or waste water resources.

While much research has gone into investigating algal lipids and biodiesel production through past programs such as the National Renewable Energy Laboratory (NREL) Aquatic Species Program (Sheehan et al. 1998) and current individual and corporate research (Mata et al. 2010), little research has focused on the algal proteins and their potential for use in human and animal foods, plastics, adhesives, and other applications (Chisti 2007; Becker 2007; Chronakis 2001). Protein is a major organic constituent of algae, and for some species, up to 71% of algae total mass can be accumulated as proteins (Becker 1994; Brown 1990; Brown and Jeffery 1992; Brown et al. 1997). Therefore, utilizing the proteins to create value-added products will increase the economic viability of algal biodiesel as well as benefit the bioindustry as a whole.

The objectives of this research were to evaluate the physical and chemical properties of algal proteins for bio-based adhesives application and increase the adhesion performance of algae proteins through chemical modification. Previous research has successfully utilized sodium hydroxide (NaOH) and sodium dodecyl sulfate (SDS) as modifiers to improve adhesion strength, water resistance, and other characteristics of soy proteins (Kumar et al. 2002; Huang and Sun 2000; Zhong et al. 2001; Hettiarachchy et al. 1995; Kalapathy et al. 1996). In this

research, algal proteins extracted from *Cladophora* sp. were modified with NaOH and SDS to improve their adhesive performance.

Chapter 2 - Literature Review

2.1 Biology of *Cladophora* sp.

Cladophora is a genus of algae that includes an estimated 180 – 1000 species of both freshwater and marine varieties (Guiry and Guiry 2012). In addition to being native to Kansas, freshwater *Cladophora* appears to be one of the most abundant types of algae in streams, rivers, and ponds around the world (Blum 1956). This section discusses the biology of *Cladophora*, with an emphasis on characteristics relevant for industrial, large-scale growth of algae biomass.

2.1.1 Morphology

Cladophora is characterized by a thallus of uniseriate branched filaments; however, the degree of branching varies greatly within the genus (Guiry and Guiry 2012; Whitton 1970). On one end of the spectrum, *C. basiramosa* displays infrequent branching while on the other end, *C. aegagropila* is densely branched and grows in thick mats or balls (Whitton 1970). Growth of *Cladophora* is both intercalary and apical (Whitton 1970). Cells are multinucleate, with the nuclear membrane remaining intact during nuclear division (Guiry and Guiry 2012). Chloroplasts of *Cladophora* are parietal and can be well separated or combined in a reticulum (Guiry and Guiry 2012). Studies have shown that some *Cladophora* species have an unusual multilayered cell wall structure that includes an amorphous inner layer and a protein-rich microfibrillar outer layer (Hanic and Craigie 1969; Whitton 1970).

2.1.2 Reproduction

Cladophora strains can reproduce sexually or asexually. Sexual reproduction is achieved by regularly alternating generations of biflagellate isogametes and quadriflagellate zoospores (Whitton 1970). Alternately, other species reproduce asexually by thallus fragmentation (Guiry and Guiry 2012).

2.1.3 Growth conditions

Cladophora can grow in a variety of conditions, and the optimal growth conditions are heavily species-dependent. The known growth conditions across the genus are discussed below.

Temperature: *Cladophora* has been observed in a variety of climates, ranging from peri-Artic to tropical. Due to difficultly in isolating growth conditions when observing *Cladophora*

in native environments, no firm maximum and minimum temperatures have been determined. However, for one extreme example, *C. glomerata* has been observed growing at water temperatures as high as 40ºC and as low as 2ºC with maximum growth near 20ºC (Whitton 1970).

Light: Most *Cladophora* species have been shown to favor high light intensities, with species such as *C. glomerata* displaying larger cell diameters and increased branching when exposed to direct sunlight (Whitton 1970). However, *C. basiramosa* and *C. aegagaropila* are examples of two species that have been found to thrive in shady areas, deep water, or other low-light conditions (Whitton 1970).

Water movement: Multiple studies have demonstrated the importance of water movement for growth of *Cladophora* (Fritsch 1906; Neil and Owen 1964; Whitford and Schumacher 1961). Neil and Owen (1964) noted that *Cladophora* in the Great Lakes proliferates only in areas with the greatest water movement, such as in current lanes. Whitford and Schumacher (1961) theorized that the increased growth of *Cladophora* in areas of greatest water flow rates is due to increased exposure and more efficient use of nutrients in the water.

pH: *Cladophora* species tend to grow in relatively high pH conditions. Few species can survive at pH 7 or lower, and *C. glomerata* has been shown to die at pH values less than 7.0 and greater than 10.0 (Bellis 1968).

Ions: *Cladophora* has been shown to favor hard water conditions, particularly those rich in calcium and magnesium (Whitton 1970). Additionally, growth of *Cladophora* is enhanced by phosphate and nitrate, which has led to nuisance growth of *Cladophora* in lakes and streams with high phosphate and nitrite loading due to urban and agricultural runoff (Whitton 1970). For all these nutrients, no upper limit of concentration has been established. On the other hand, heavy metals, such as zinc and copper, are toxic to many species of *Cladophora*, causing the cell wall to display large, colorless bladders (Whitton 1970). Table 2.1 summarizes the known maximum and minimum ion concentrations for *Cladophora* growth.

Table 2.1 Minimum and maximum ion concentrations required for survival of *Cladophora* (Whitton 1970)

	Concentrations for survival, mg/L	
Ion	**Minimum**	**Maximum**
Calcium	1.2	None Observed
Magnesium	0.7	None Observed
Phosphate	0.07	None Observed
Nitrate	0.6	None Observed
Ammonium	0.2	None Observed
Zinc	None Observed	0.05
Copper	None Observed	0.1

2.1.4 Chemical composition

Little comprehensive research on the chemical composition of *Cladophora* species has been performed. However, Maddi et al. (2011) reported that a *Cladophora* sp. collected from Lake Erie (New York, USA) was composed of 24.8% carbohydrates, 5.8% lipids, 24.6% protein, and 13.3% ash by mass. Kutsin and Tkachenko (2011) analyzed the lipid fraction of *Cladophora vagabunda* in greater depth, finding that fatty acids accounted for nearly 83% of the lipids and oleic and linoleic fatty acids were most abundant.

2.1.5 Interaction with other organisms

Although monoculture of *Cladophora* species at the laboratory scale has been achieved and maintained for long periods of time, *Cladophora* displays a high level of interaction with other organisms in native (or simulated) environments (Whitton 1970). Commonly reported epiphytes include: bacteria, *Chamaesiphon, Oedogonium, Diatoma elongatum, Cocconeis pediculus,* and *C. placentula* (Whitton 1970). Researchers have assumed that dense coverings of such epiphytes, particularly diatoms, would interfere with nutrient uptake by *Cladophora* and therefore inhibit growth; however, no quantitative data exists to test this hypothesis. *Cladophora* may also compete with other macrophytes, such as *Rannculus fluitans, Fontinalis antipyretica,* and *Stigeoclonium,* for light exposure, attachment substrate, and nutrients (Whitton 1970). *Cladophora* has not been reported to be sensitive to parasitic infections by bacterial, viral, or fungal agents (Whitton 1970).

2.2 Algae for biodiesel

The use of algal biomass for the production of biodiesel is the subject of much research. This section provides a background for this technology and proposes a production strategy that includes utilizing algal proteins to develop co-products such as algal protein-based adhesives.

2.2.1 Brief historic perspective

The concept of large-scale algae production for use as a commodity is not new. As early as World War II, German scientists conceived of cultivating algae in open ponds for use as a food protein source (Soeder 1986). In the United States in 1951, Arthur D. Little of Carnegie Institute constructed and operated a *Chlorella* pilot plant for food protein production (Burlew 1953). The use of algae as a fuel source first arose in a much different form than biodiesel. In 1955, Meier proposed that algal carbohydrates could be used to produce methane gas and by 1960, Oswald and Golueke had proven the concept of using anaerobic digestion to produce methane from algae biomass on a lab scale. Major developments in algal biodiesel didn't begin until the creation of the Department of Energy's Aquatic Species Program (ASP) in 1978 (Sheehan et al. 1998). As part of the ASP, the National Renewable Energy Laboratory (NREL) investigated algal strain characterization, physiology, biochemistry, genetic engineering, process development, and culturing with an emphasis on evaluation of lipid production for biodiesel (Sheehan et al. 1998). The ASP also performed pilot-scale open pond algae cultivation and detailed resource assessments to demonstrate the availability of adequate land, water, and CO_2 resources in the southwestern United States for large scale algae cultivation and biodiesel production (Sheehan et al. 1998). Following the discontinuation of the ASP in 1996 due to lack of funding, research into algae for biodiesel has waxed and waned in the past decade as the price of petroleum has fluctuated (Hu et al. 2008). Current research areas include engineering challenges such as harvesting methods, genetic engineering for greater lipid accumulation, and optimizing processing conditions (Scott et al. 2010).

2.2.2 Overview of algae biodiesel production

This section discusses the production of algae biodiesel, including cultivation, harvesting, processing, and utilization of co-products in a biorefinery production model. Figure 2.1 illustrates the basic algae biodiesel production process.

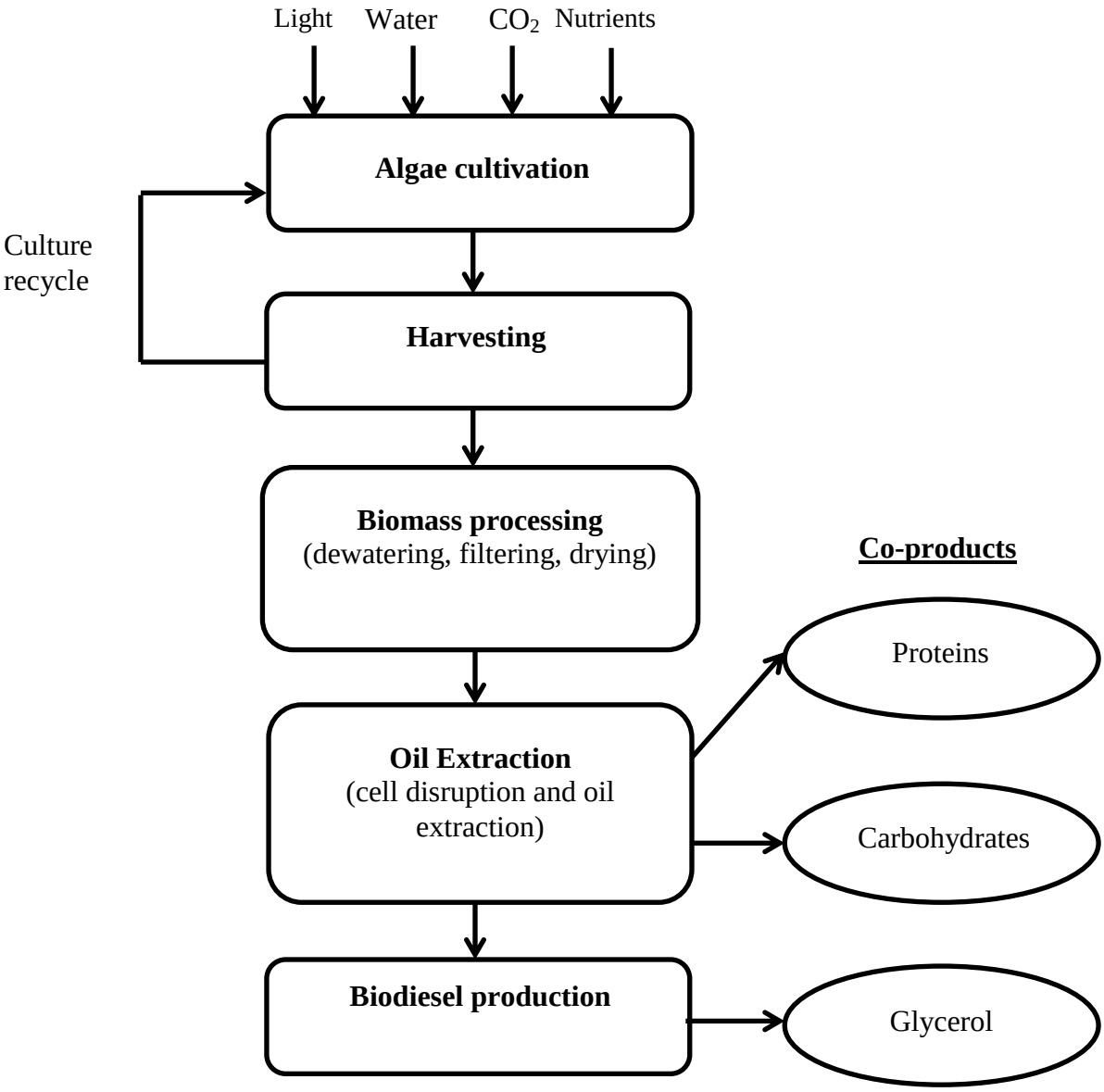

Figure 2.1 Flowchart of algae biodiesel production

The following sections describe each of the steps represented in Figure 2.1 in detail.

2.2.2.1 Algae cultivation methods

The two basic methods of large-scale algae cultivation are open ponds and closed photobioreactors. While open pond cultivation requires less energy and has lower capital cost, photobioreactors have the potential to produce larger quantities of algal biomass and minimize contamination.

Although many designs of open ponds for algae cultivation exist, the most widely used for large-scale production is the raceway pond (Chisti 2007). An illustration of a raceway pond is shown in Figure 2.2.

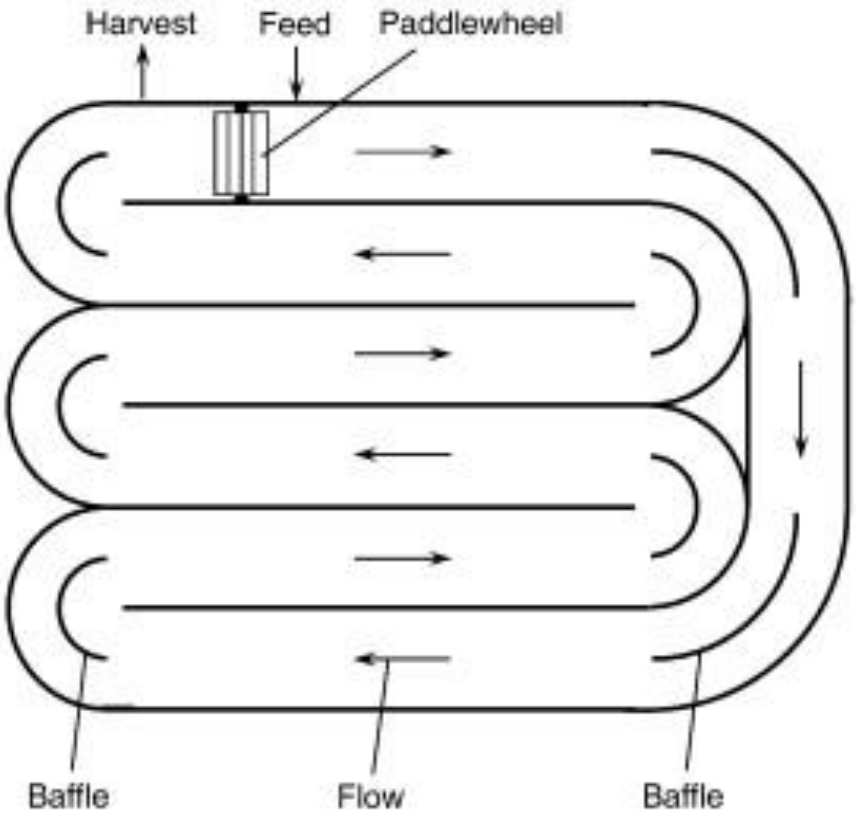

Figure 2.2 Arial view of a raceway pond. (Chisti 2007)

A raceway pond is a shallow, closed-loop channel that uses a paddlewheel to provide circulation. The ponds are commonly built out of concrete or compacted earth and often have a white liner to increase light distribution (Chisti 2007). Because the pond is open, it is subject to temperature fluctuations, evaporative water loss, and contamination from unwanted algae strains and other microorganisms (Sheehan et al. 1998). Due to these factors, algal biomass production in open ponds is low when compared to photobioreactors. However, the low capital and operating costs of open ponds makes them an attractive option for large-scale algae production for biodiesel (Sheehan et al. 1998).

Photobioreactors are closed systems, often composed of transparent tubes, in which a single species of algae is cultured. Many designs of tubular photobioreactors have been

developed, but most designs utilize similar components including, transparent tubes less than 10 cm in diameter arranged to maximize solar collection, a degassing column, a pump, a cooling system, and a harvesting mechanism (Chisti 2007). One example of a photobioreactor is shown in Figure 2.3.

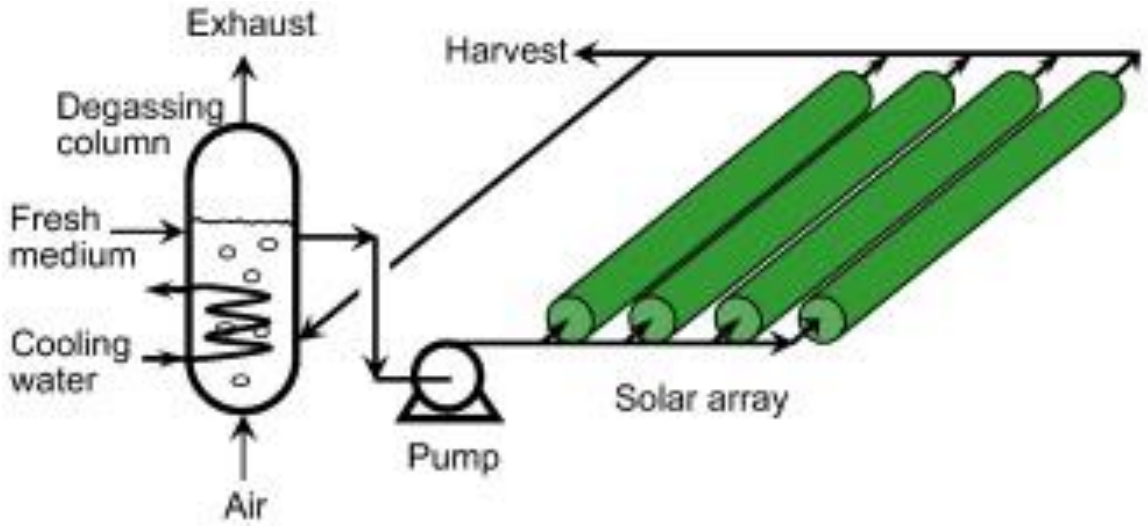

Figure 2.3 Tubular photobioreactor. (Chisti 2007)

Construction and operation of photobioreactors is considerably more expensive and energy intensive than open ponds (Sheehan et al. 1998); however it offers the advantage of greater algal biomass production due to the ability to limit contamination (Chisti 2007).

2.2.2.2 Harvesting

Development of methods of harvesting algal biomass is an area of ongoing engineering research, and no universal harvesting system currently exists (Mata et al. 2010). This is due to the need for multiple steps in the harvesting process, as well as due to the variety of algal species that require unique and economical harvesting solutions. Some common harvesting methods include sedimentation, flocculation, centrifugation, filtration, and flotation with float collection (Mata et al. 2010).

2.2.2.3 Biomass processing and component extraction

Following harvesting, the algal biomass is typically dried to increase shelf life (Scott et al. 2010). Many methods of drying can be used, including spray-drying, drum-drying, and sun-drying (Richmond 2004).

Once the algae are dry, the cells must be disrupted to release the lipids for biodiesel production. Cell disruption methods vary according to the properties of the algal species used. Some common methods of cell disruption are cell homogenizing, bead milling, ultrasounds, autoclaving, freezing, organic solvents, and enzyme reactions (Mata et al. 2010).

After cell disruption, the lipids must be extracted. Solvent extractions using hexane or ethanol are common, and can yield up to 98% extraction of fatty acids (Richmond 2004). Caution must be exercised during lipid extraction to avoid contamination by other cellular components, such as DNA and chlorophyll (Scott et al. 2010).

2.2.2.4 Biodiesel production

Biodiesel is produced by the transesterification of triglycerides. Following oil extraction, the triglycerides in the lipids are reacted with methanol in the presence of a catalyst, commonly sodium hydroxide, to form methyl esters (biodiesel) and the co-product glycerol. Figure 2.4 illustrates the transesterification reaction.

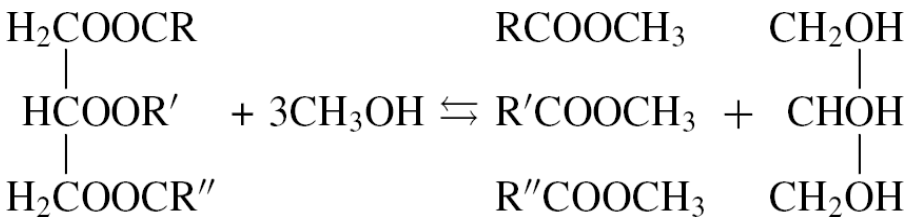

Figure 2.4 Diagram of transesterification of oil to biodiesel. R-R" are hydrocarbon groups.

This reaction is commonly carried out at 60 ºC and atmospheric pressure, and can be completed in about 90 minutes or less under these conditions. In industrial biodiesel production, it is common to use 6 mol of methanol per mole of triglyceride in order to drive the reaction toward the production of methyl esters (Fukada et al. 2001). With conventional oil feed stocks, this method is reported to achieve greater than 98% yield of methyl esters on a weight basis (Fukada

et al. 2001). Following the reaction, the biodiesel is recovered by multiple washings with water to remove methanol and glycerol.

2.2.3 Comparison of algae to conventional oilseed crops

At the current rate of consumption, replacing all of the transportation fuel consumed in the United States with biodiesel will require more than 0.5 billion m^3 of biodiesel annually (Chisti 2007). However, current sources of oil for biodiesel production, such as animal fats, waste cooking oil, and oilseed crops, simply cannot meet this demand (Chisti 2007). Additionally, conventional oilseed crops, including soybean and canola, require arable land and fresh water for production and are used as a human feedstock (both directly and indirectly). Using these crops for fuels would result in competition between energy and food production that can drive up the cost of both food and fuel (Mata et al. 2010). Algae, on the other hand, can utilize waste or brackish water and marginal lands for growth, and doesn't compete with the food supply (Chisti 2007). Most importantly, algae can produce up to 300 times more oil per unit growth area than soybeans (Chisti 2007). Major studies, such as those conducted by the Department of Energy as part of its Aquatic Species Program, have shown that land, water, and CO_2 resources in the United States can support substantial biodiesel production from algae, while other oil crops fall short (Sheehan et al. 1998; Chisti 2007).

2.2.4 Biorefinery strategy to improve economics of algal biodiesel

Although large-scale algal biodiesel production is feasible from technological and resource availability perspectives, the cost of producing algal biodiesel is not yet economically competitive with petroleum diesel (Mata et al. 2010; Chisti 2007). While technological advances are expected to reduce the production cost in the coming years (Scott et al. 2010; Chisti 2007), a more fundamental approach can be used to lower production costs – the biorefinery (Chisti 2007). A biorefinery, similar to conventional petroleum refineries, would utilize every component of the raw biomass to produce valuable products (Chisti 2007). By using all components of the biomass, the cost of producing any one product is lowered and the impacts of market fluctuations can be decreased. Currently, only a few options for value-added co-products from algal biodiesel production residues have been explored. These include the well-established production of glycerol for pharmaceuticals and cosmetics, use of algal proteins for animal feeds, and production of methane by anaerobic digestion of residual biomass (Chisti 2007). However,

these products, with the exception of glycerol at high purity levels, are low value commodities. Development of high-value co-products from algal biodiesel production residues is needed to further increase the economic feasibility. One example of a potentially high-value co-product is algal protein-based adhesives. As described further in Research Objectives, the purpose of this research is to develop and evaluate algal protein-based adhesives.

2.3 Algae proteins

Although the concept of large-scale production of algae for protein has existed since the 1940s, relatively little research has been conducted on algal proteins. Rather, the majority of research about algae has focused on lipids and issues related to lipid production. The existing research on algal proteins focuses primarily on algal proteins for human food and animal feeds, and this research is summarized in the following sections.

2.3.1 Chemical composition of algae

The chemical compositions of many different algae strains have been studied. The macromolecular composition of algae varies widely depending on algal strain and environmental conditions. Becker (2007) reported that protein content of different algae strains ranges from 6% (dry matter) for *Spirogyra* sp. to 71% for *Arthrospira maxima*. Additionally, carbohydrate content varies from 8% to 64% (dry matter) for *Spirulina platensis* and *Spirogyra* sp., respectively, and lipid content varies from 2% in *Chlorella pyrenoidosa* to 22% in *Chlorella vulgaris* (Becker 2007).

2.3.2 Properties of algal proteins

Very little is known about the properties of algal proteins. In general, the amino acid profiles of algal proteins vary from species to species, but several algal strains contain all the essential amino acids (Becker 2007). A study of the functional properties of *Spirulina platensis* for food applications by Chronakis (2001) is one of the best sources of information available on algal protein properties. Chronakis (2001) found that thermal transitions of *Spirulina platensis* take place near 67 and 109 ºC at neutral pH; however, calcium chloride stabilized the quaternary structure and increased the thermal stability of the proteins. Additionally, the viscosity of *Spirulina platensis* protein was shown to be dependent on pH. At high pH (>8), the viscosity of the protein decreased due to increased solubility (Chronakis 2001). Furthermore, strong

hydrophobic protein-protein interactions, as well as hydrogen bonding and intermolecular disulfide bonds, were found to impact the thermal gelation behavior of the proteins (Chronakis 2001).

2.3.3 Current applications of algal proteins

Currently, algal proteins are primarily used as food products, both for human consumption and animal feed. Algal protein, both in the form of whole algae and as various levels of protein concentrate, is currently used in some human foods. Predominantly, algal proteins are marketed as "health foods." Algal proteins have been blended into juice drinks or encapsulated for consumption (Becker 2007). While researchers have investigated adding algal protein to conventional foods, such as breads and noodles, the consistency, color, and taste of algal biomass was generally found not to be palatable. In addition to the unfavorable taste, algal protein production costs are currently higher than most conventional protein sources, making the use of algal protein in human foods quite rare (Becker 2007). In recent years, algal biomass has increasingly been incorporated into animal feeds as a means to increase protein content. Estimates state that nearly 30% of current world algal production is sold for animal feed (Becker 2007). The majority of algal protein for animal feed is used for poultry and fish (Becker 2007).

2.4 Soy proteins for adhesives

Soybeans are the largest source of edible oil and account for over half of the world's oilseed production (Kumar et al. 2002). The soy proteins as co-products from soy oil production are utilized to produce a variety of products, including human food and animal feeds, bioplastics, adhesives, and composites (Kumar et al. 2002). Currently, soy protein is one of the largest sources of renewable adhesives, and soy-based adhesives represent a growing share of the total adhesive market (Kumar et al. 2002). The following section reviews the properties of soy protein and discusses their use as adhesives. Due to the abundance of research into soy proteins for adhesives, soy proteins have been chosen as a model for development of algal protein-based adhesives in this study.

2.4.1 Properties of soy protein

The majority of soy proteins are globulins with an isoelectric region of pH 4.2 – 4.6 (Kinsella 1979). Soy protein fractions are often characterized by their sedimentation constants in

water at 20ºC. Soy protein contains two major fractions, 7S and 11S (S stands for Svedburg units), and two minor fractions, 2S and 15S. Soy proteins contain approximately 52% of 11S, 35% of 7S, 8% of 2S, and 5% of 15S (Kinsella 1979). The 2S fraction includes low molecular mass polypeptides (8000-20,000 Da), such as soybean trypsin inhibitors. The 7S fraction is more heterogeneous, but it is primarily composed of beta-conglycinin, hemagglutinins, and enzymes, such as beta-amylase and lipoxygenase. The 11S fraction largely consists of glycinin, with a quaternary structure composed of three acidic and three basic subunits stabilized by disulphide bonds. The 15S fraction is thought to be a dimer of glycinin (Kinsella 1979).

2.4.2 Types of soy protein products

Soy protein materials come in a variety of forms. These include soy flour, defatted soy meal, soy protein concentrates, and soy protein isolates. Soy flour, protein concentrate, and protein isolate are especially useful for adhesives (Kumar et al. 2002). Soy flour contains less than 1% oil and has a protein content of 40-60% while soy protein concentrate contains 65-75% protein and soy protein isolate contains 96-98% protein (Kinsella 1979).

2.4.3 Performance of soy protein adhesives

The performance of soy protein adhesives is determined by several characteristics, including particle size, viscosity, protein structure, and processing conditions (Kumar et al. 2002). Metrics of soy protein adhesives include shear strength, water resistance, and shelf life.

Soy protein particle size can impact the performance of the adhesive. For adhesives, a specific surface area of 3000-6000 cm^2/g is considered acceptable (Lambuth 2001).

The viscosity of the soy protein adhesive is an important property (Lambuth 1977). Soy proteins tend to have high viscosities due to the intermolecular interactions of unfolded proteins, such as disulphide bonds and electrostatic forces (Hettiarachchy and Kalapathy 1998). Because ions can weaken the electrostatic interactions between protein molecules, the viscosity of protein can be adjusted by adding salts or reducing agents. Additionally, alkaline hydrolysis reduces viscosity, but excessively high pH reduces storage life. Accordingly, a moderate pH of 10 is recommended for acceptable protein viscosity (Kalapathy et al. 1996).

Protein structure can affect adhesive performance by determining availability of apolar and polar groups of proteins for interaction with the wood material. In native protein, van der Waals forces, hydrogen bonds, and hydrophobic interactions prevent access to most polar and

apolar groups; however, alkali hydrolysis can unfold the protein molecules to allow access to polar and apolar groups and improve adhesive performance (Lambuth 1977).

Many processing conditions, such as pre-pressing drying time, pressing time, pressing temperature, and protein concentration, can affect the performance of soy protein adhesives. Zhong et al. (2001) observed an increase in shear strength as pressing time increased from 1 to 10 minutes and as pressure increased from 0.4 to 4 MPa. Increasing pressing temperature from 25 to 100ºC also increased shear strength. Additionally, shear strength was impacted by protein concentration, with maximum shear strength occurring at a soy protein: water ratio of 12:100 (w/w).

2.4.4 Soy protein adhesive modifications

A variety of modifications were used to improve the performance of soy protein adhesives. Four of the basic categories of modification are 1) denaturation of proteins, 2) crosslinking, 3) enzyme modifications, and 4) chemical reactions (Kumar et al. 2002).

Proteins can be denatured using acids, alkalis, organic solvents, detergents, and urea. Alkali modifications of soy protein adhesives have been studied extensively, and are reported to increase shear strength and water resistance (Hettiarachchy et al. 1995). Hettiarachchy et al. (1995) proposes a pH value of 10 to increase shear strength while minimizing staining and other undesired effects. Alkali modification is most commonly achieved with sodium hydroxide (Kumar et al. 2002). Sodium dodecyl sulphate (SDS) modification (0.5 – 1%) has also been shown to increase shear strength and water resistance by denaturing soy proteins (Huang and Sun 2000). SDS modification also eliminated delamination (Huang and Sun 2000).

Crosslinking compounds can be used to increase the water resistance and consistency of soy protein adhesives. Common crosslinkers include sulphur compounds, such as CS_2 and thiourea, and formaldehyde donors, such as dimethylol urea and sodium formaldehyde bisulphite (Kumar et al. 2002). Very low concentrations (0.1-1%) of crosslinkers are required for enhancing water resistance and shelf life (Kumar et al. 2002).

Enzyme modification can be used to increase soy protein solubility and emulsifying properties (Kumar et al. 2002). Enzymes modify proteins largely through cleavage of peptide bonds. Enzymes utilized include pepsin, trypsin, chymotrypsin, papain, and pronase (Kumar et al. 2002).

Proteins can be modified with chemical reactions, notably succinylation and acetylation, in order to improve properties such as solubility and surface hydrophobicity (Kumar et al. 2002). Soy proteins modified by succinylation and acetylation have been used as a binder for paper coating application (Kumar et al. 2002).

2.5 Research objectives

The goal of this research was to develop value-added products from algae biodiesel production residues to improve the economic attractiveness of algal biodiesel production. The specific objectives were 1) to characterize the chemical and physical properties of algal protein; 2) to evaluate the potential of algal proteins for protein-based adhesives; and 3) to increase the adhesion performance of algae proteins through chemical modification.

Chapter 3 - Materials and Methods

3.1 Materials

1M Sodium hydroxide and 6M hydrochloric acid were obtained from Fisher Scientific (Pittsburgh, PA, USA). Sodium dodecyl sulfate and 2-mercaptoethanol were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cladophora sp. algae samples were collected with a net from a 400 m^2 experimental pond at the University of Kansas Field Station (Lawrence, KS, USA) care of Dr. Val Smith (Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS, USA). Algae were then gently centrifuged at 3000 rpm to remove extracellular water and air-dried for 12 h with forced air at room temperature. Air-dried *Cladophora* was cleaned by hand to remove large contaminants and oven-dried at 50ºC. *Cladophora* samples were subsequently milled using a cyclone sample mill (Udy Corporation, Fort Collins, CO, USA) with a 0.25 mm screen. Milled samples were stored at 4ºC for future use. Alternatively, *Cladophora* samples prepared for chemical composition analysis were washed three times with distilled water following hand-cleaning, then centrifuged at 3000 rpm to remove excess water, oven-dried, and milled following the procedures described above.

3.2 Methods

3.2.1 Protein extraction

Protein was extracted from *Cladophora* samples using a procedure adapted from Barbarino and Lourenço (2005). Five grams of algae was mixed with 40 mL of distilled water and allowed to soak for 24 h with gentle agitation at 150 rpm in order to soften the outer thalli of the algae. A French press (Thermo Electron Corporation, Neednam Heights, MA, USA) was used to disrupt the algae cells in the aqueous mixture. Samples were pressed twice at 17 MPa to ensure maximum cell disruption. Samples were then centrifuged at 8,000 rpm and 4ºC for 20 min. The supernatant fraction was saved. Sodium hydroxide (0.1 N) with 0.5% 2-mercaptoethanol (100 mL) was added to the pellet, and the pellet solution was solubilized for 24 h with agitation at 150 rpm. The suspended pellet was centrifuged at 8,000 rpm and 4ºC for 20 min and alkaline supernatant was combined with reserved aqueous supernatant. Protein was precipitated by adding cold 25% HCl to supernatant at 25% HCl to supernatant ratio of 2.5:1

(v/v) and placing in an ice bath for 12 h. The protein precipitate was separated by centrifugation at 8,000 rpm and 4ºC for 20 min. The pellet fraction was washed with 10% HCl to rinse out acid soluble carbohydrates and then centrifuged at 8,000 rpm and 4ºC for 20 min. Pellet rinsing and centrifugation were repeated using 5% HCl and distilled water. After rinsing, protein extract (pellet) was frozen in 10 mL of distilled water and freeze-dried. Figure 3.1 is a flowchart of this extraction process.

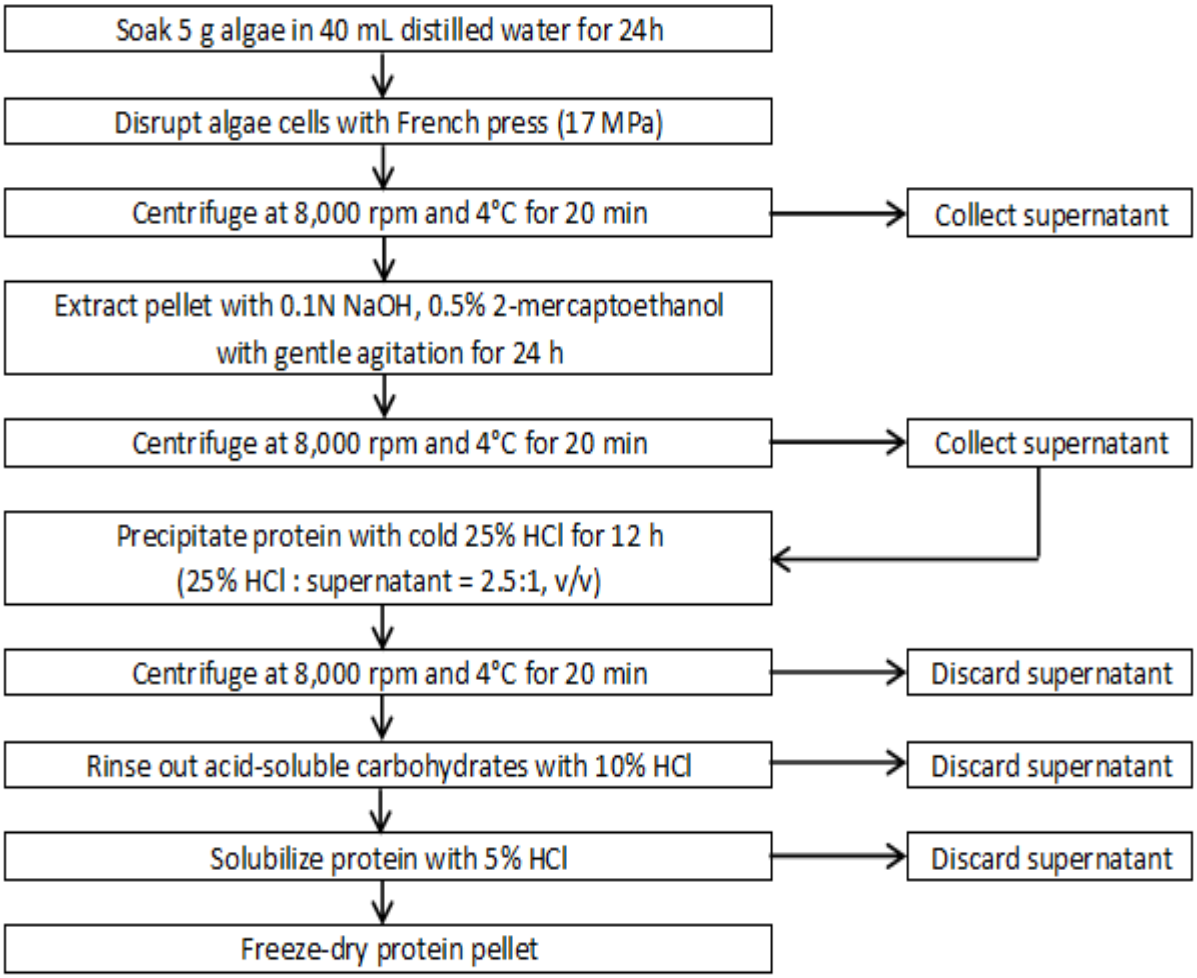

Figure 3.1 Flowchart of algae protein extraction procedure

3.2.2 Chemical composition

3.2.2.1 Macromolecular analysis

Crude protein, total starch, and fatty acid profiles of whole algae and algae protein extract were determined using AOAC Official Method 990.03, AOAC Official Method 979.10, and the Palmquist method, respectively. Crude protein was calculated from crude nitrogen by multiplying by a 4.92 conversion factor, as recommended by Lourenco et al. (2002). Reported values are the averages of three replications.

3.2.2.2 Amino acid analysis

Amino acid content of algae protein extract was determined using acid hydrolysis. Approximately 100 mg of sample for protein extraction were weighed and then placed in about 0.5 mL of 6 N HCl along with the internal standard and were hydrolyzed at 110 °C for 20 h. An aliquot, typically 10 or 20 µl, was then made up to 250 µl with 0.4 M borate buffer to dilute the sample and raise the pH. After precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 µl of this diluent was injected into an HPLC system with a C18 column (Hypersil AA-ODS, 2.1 × 200 mm, 5 µm). Mobile phase A was 20 mM sodium acetate buffer with 0.018% (v/v) triethylamine, 0.05 mM EDTA, 0.3% tetrahydrofuran, and pH adjusted to 7.2 using acetic acid. Mobile phase B was 100 mM sodium acetate:acetonitrile:methanol (20:40:40, v/v). The elution conditions went from 100% A to 60% B in 17 min at 0.45 mL/min. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

3.2.3 Protein modification

Alkali-modified and sodium dodecyl sulfate (SDS) -modified algae proteins were prepared. As a standard for comparison, unmodified algae protein samples were also prepared by suspending 1g of protein extract in 10 mL of distilled water and stirring for 10 min until uniformly dispersed.

3.2.3.1 Alkali-modified algae protein

Alkali-modified algae protein (AMAP) was prepared using the method utilized to modify soy protein (Hettiarachchy et al. 1995). One gram of *Cladophora* protein extract was suspended in 10mL of distilled water and stirred for 10 min to achieve uniform dispersion. Each suspension was then adjusted to a pH of 9, 10, or 11 using 1N NaOH. The mixture was heated to 50ºC and stirred for 1 h. If AMAP was not used immediately, the sample was freeze-dried and milled into a powder. AMAP powder was reconstituted by mixing 1g AMAP with 10 mL distilled water and mixing at room temperature until well dispersed.

3.2.3.2 SDS-modified algae protein

SDS-modified algae protein was prepared using the method developed for soy protein (Huang and Sun 2000). Solutions of SDS at concentrations of 0.5, 1, and 3% were prepared at room temperature. *Cladophora* protein extract (1g) was suspended in each SDS solution (10 mL) and stirred for 6 h.

3.2.4 Shear strength measurements

Wood specimen preparation was performed following the method described by Qi et al. (2012), and shear strengths, including dry strength, wet strength, and soaked strength, were performed following ASTM Standard Methods D2339-98, D1151-00, and D1183-03, respectively (ASTM 2002). Cherry wood veneers with dimensions of 50 x 127 x 5 mm were conditioned for 7 days at 23ºC and 50% relative humidity (RH) in an environment chamber (Electro-Tech Systems, Inc., Glenside, PA, USA). The modified and unmodified algae proteins (control) were brushed onto one end of a wood veneer with dimensions of 127 x 20 mm (length x width) until entire area was completely wet. Amount of protein mixture on each veneer was controlled with a balance. Two brushed wood veneers were immediately assembled, as shown in Figure 3.2, and conditioned for 15 min at room temperature.

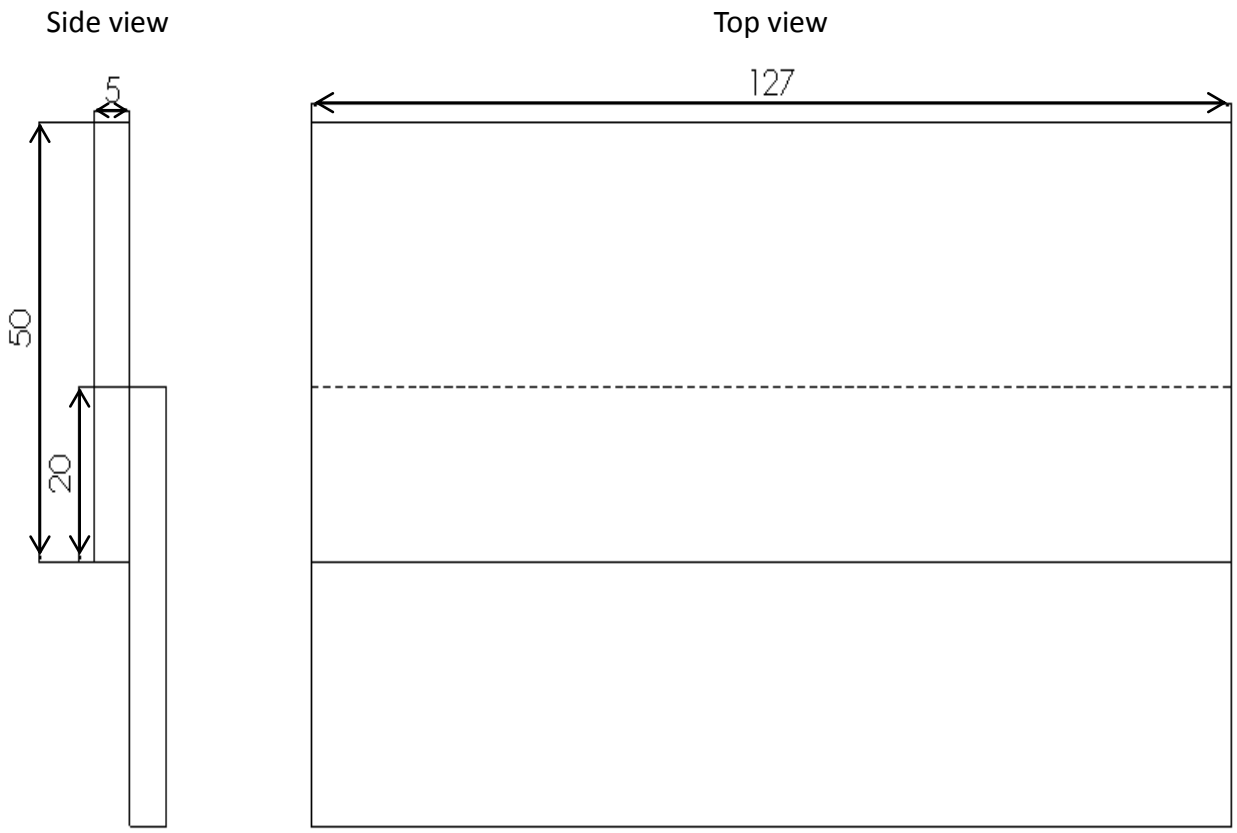

Figure 3.2 Side and top view of wood specimen assembly. All dimensions in millimeters.

Assembled wood specimens were pressed at 1.4 MPa and 150ºC for 10 min with a hot press (Model 3890 Auto M; Carver, Inc., Wabash, IN, USA). The wood assemblies were conditioned at 23ºC and 50% RH for 48 h and then cut into five pieces with overall dimensions of 80 x 20 mm and glued dimensions of 20 x 20 mm. Dimensions of cut wood specimens are shown in Figure 3.3.

The cut wood specimens were conditioned for 4 additional days at the same conditions before testing. Shear strength testing was performed using an Instron (Model 4465; Canton, MA, USA) at a crosshead speed of 1.6mm/min according to ASTM Standard Method D2339-98 (ASTM 2002). Shear strength at maximum load was recorded. Values reported are the average of five specimen measurements.

Water resistance of the cut wood assemblies was tested by soaking preconditioned specimens in tap water at 23ºC for 48 h and immediately testing wet shear strength at maximum load according to ASTM Standard Methods D1183-03 and D1151-00 (ASTM 2002).

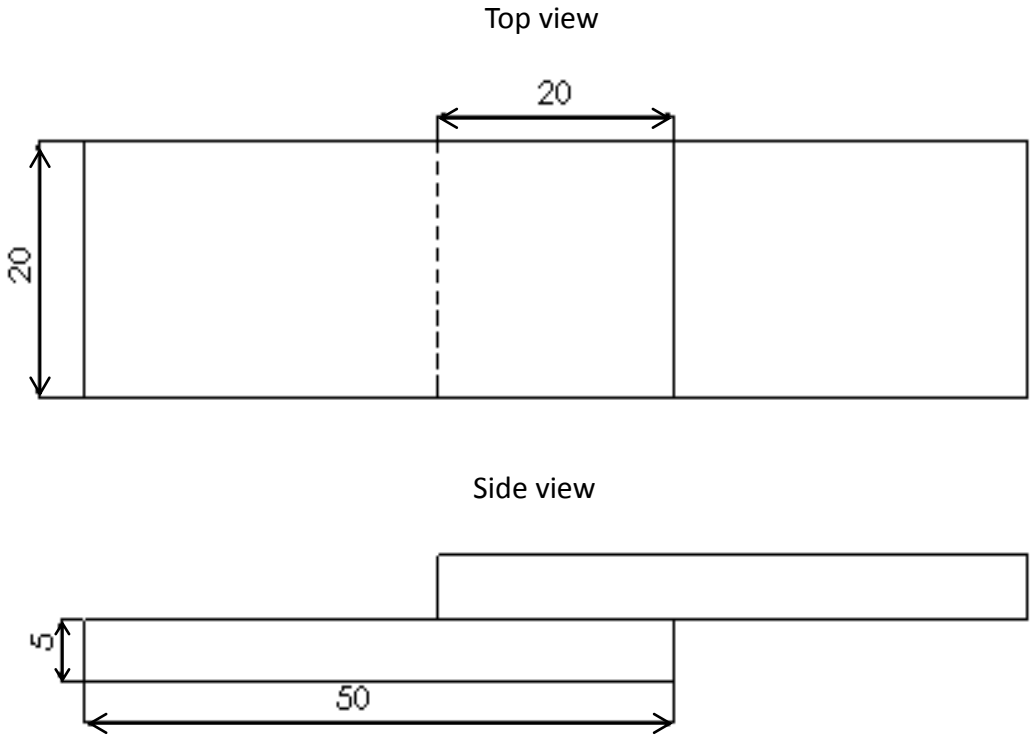

Figure 3.3 Top and side views of cut wood specimen. All dimensions in millimeters.

3.2.5 Rheological properties

Rheological properties of modified and unmodified algae proteins were determined using a Brookfield programmable rheometer (DV-III +) equipped with a Small Sample Adapter (SC4-21/13R; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) according to the method described by Zhong et al. (2001). Rheological properties were recorded as functions of time, temperature, and shear rate. Time-dependence was measured at a constant shear rate of 140 s^{-1} at 60 s intervals for 15 minutes. Temperature-dependence was measured at the same shear rate as the temperature increased from 30ºC to 90ºC at a rate of 5 ºC/min. Shear rate-dependence was measured as the shear rate increased to 230 s^{-1}.

3.2.6 Differential scanning calorimetry

Thermal properties of modified and unmodified algae proteins were measured using differential scanning calorimetry (DSC) (DSC 7; PerkinElmer, Norwalk, CT, USA) according to the method described by Huang and Sun (2000). Samples were placed in large DSC pans with excess water in a sample to water ratio of 1:10. Pans were sealed, and samples were equilibrated at 4ºC overnight. Samples were then equilibrated to 0ºC by the DSC and heated to 150ºC at a heating rate of 10 ºC/min. Values reported are the average of two replications.

3.2.7 Scanning electron microscopy

Morphology of modified and unmodified algae protein samples was observed by scanning electron microscopy (SEM) (Hitachi S-3500 N; Hitachi Science System, Ibaraki, Japan). Samples were freeze-dried, ground into a fine powder, and placed on an aluminum stage using two-sided adhesive tape. Samples were sputter-coated with an alloy of 60% gold and 40% palladium (Desk II Sputter/Etch Unit, Moorestown, NJ, USA). SEM imaging was performed at an accelerating voltage of 20kV.

Chapter 4 - Results and Discussion

4.1 Chemical composition

4.1.1 Chemical composition of algae

The whole algae was composed of 27.2% crude protein, 6.26% starch, and 3.10% fatty acids (dry weight). This is consistent with reported compositions of *Cladophora* sp. (Maddi et al. 2011).

The fatty acids consisted of 42.3% saturated fatty acids, 12.2% mono-unsaturated fatty acids, and 45.5% poly-unsaturated fatty acids. Palmitic acid (33.2%), oleic acid (4.3%), and α-linolenic acid (30.8%) are the most abundant saturated, mono-unsaturated, and poly-unsaturated fatty acids, respectively. Important characteristics of biodiesel quality, including cetane number (ignition quality), cold-flow properties, and oxidative stability, are largely determined by the feedstock's fatty acid components (Knothe 2005). Saturated fats produced biodiesel with greater oxidative stability and a higher cetane number, but poor low temperature properties that can cause gelling at ambient temperatures. On the other hand, poly-unsaturated fatty acids tend to produce biodiesel with good cold-flow properties but are prone to oxidation (Hu et al. 2008). Because *Cladophora* has a relatively balanced ratio of saturated to poly-unsaturated fatty acids (1:1.08), it would be expected to exhibit moderate ignition quality, oxidative stability, and cold-flow properties. However, the low overall concentration of fatty acids in the algae makes it a poor candidate for biodiesel production compared to other algal species, such as *Chlorella*, except in possible environments where *Cladophora* would have significant productivity advantages (Mata et al. 2010).

4.1.2 Algae protein extracts

After extraction, the *Cladophora* sp. algae protein had a crude protein content of 40.02% (dry weight) as calculated from crude nitrogen, which represents a 47% increase in protein content from the whole algae. The rate of extraction, as defined by Equation 1, was 23.1%.

$$Extraction\,rate\,(\%) = \frac{Total\ protein\,after\,extraction}{Original\,total\ protein\,in\ algae} \times 100 \qquad (1)$$

Commercial soy protein extraction rates can be greater than 60% (Kinsella 1970). This suggests that optimizing the extraction procedure for *Cladophora* and other algae species to increase the extraction rate is needed prior to large scale production of algae protein products.

Amino acid analysis was also performed. Amino acids represent 33.43% of the dry weight of the protein extract. The discrepancy between crude protein estimated from crude nitrogen and protein calculated from the sum of amino acids can be accounted for by the errors in each method. Summing amino acids tends to underestimate protein content due to the destruction of tryptophan, cysteine, and proline during acid hydrolysis, while using crude nitrogen and a standard conversion factor causes overestimation by including some non-protein nitrogen (NPN). Lourenco et al. (2002) has developed nitrogen-to-protein conversion factors (N-Prot factors) for over 20 species of tropical marine algae, which range from 3.75 to 5.72 and recommend an average value of 4.92 for all algae, which was used for crude protein calculations in this study. However, Lourenco et al. (2002) noted that the variations among species and the overall low protein content of tropical algae make these conversion factors difficult to generalize to other algae species. In the absence of N-Prot factors specific to *Cladophora*, the sum of amino acids is considered the more accurate method of determining protein content (Lourenco et al. 2002).

The amino acid composition of the protein extract is shown in Table 4.1. The protein extract contained 54.1% (dw) hydrophilic or polar amino acids and 44.2% (dw) hydrophobic or non-polar amino acids. In comparison, soy protein is composed of approximately 65% and 35% (dw) hydrophilic and hydrophobic amino acids, respectively (Kumar et al. 2002). The comparatively high concentration of hydrophobic amino acids in the algae protein extract could contribute to the lower water solubility of algae protein extract. Difficulties in achieving uniform dispersions of unmodified and sodium dodecyl sulfate (SDS) -modified algae protein were observed, even after several hours of agitation. Solubility was significantly improved by the addition of NaOH, as observed in the alkali-modified algae protein (AMAP) samples. Studies have noted that protein from several species of algae, including *Spirulina* and *Tetraselmis*, have low solubility in water at pHs below 6 (Nirmala et al. 1992, Schwenzfeier et al. 2011). Because the pH of unmodified and SDS-modified algae protein samples were between 4.7 and 5.2, low solubility was expected.

Table 4.1 Amino acid profile of algae protein extract

Amino Acid	Amino acid fraction (% db)
Aspartate & Asparagine	11.69
Threonine	5.47
Serine	6.35
Glutamate & Glutamine	11.86
Glycine	7.64
Alanine	8.64
Valine	4.39
Methionine	1.89
Isoleucine	3.95
Leucine	11.21
Tyrosine	4.00
Phenylalanine	6.49
Histidine	2.57
Ornithine	1.68
Lysine	5.76
Arginine	6.41

4.2 Rheological properties

Rheological properties of the modified algae protein adhesives were measured as functions of time, shear rate, and temperature.

4.2.1 Time-dependent behavior

At a constant shear rate of 140 s^{-1}, unmodified algae protein and alkali-modified algae protein (AMAP) displayed time-independent behavior (Figure 4.1). On the other hand, the apparent viscosities of SDS-modified algae protein adhesives decreased with time. This thixotropic behavior became even more pronounced at higher SDS concentrations.

Additionally, the apparent viscosity of the SDS-modified adhesives increased with increasing SDS concentration, while increasing the pH of the AMAP from 9 to 11 resulted in a decrease in viscosity. SDS-modification has been reported to increase the apparent viscosity of soy protein due to swelling and unfolding of the protein molecules, which increases the hydrodynamic volume and decreases the distance between molecules (Zhong et al. 2001).

Moreover, similar results regarding increases in viscosity due to SDS-modification have been reported in the algae species *Scendesmus obliquus* and *Klebsormidium flaccidum* (Dua et al. 1993). Conversely, at high pH values, alkali modification has been shown to decrease the viscosity of soy protein due to hydrolysis of disulphide bonds and disaggregation of the soy proteins (Kumar et al. 2002; Kalapathy et al. 1996; Hettiarachchy et al. 1995; Kinsella 1979). Increasing the pH has also been shown to decrease the viscosity of algae protein isolate from *Spirulina platensis*, which is believed to be the result of changes in particle size (Chronakis 2001).

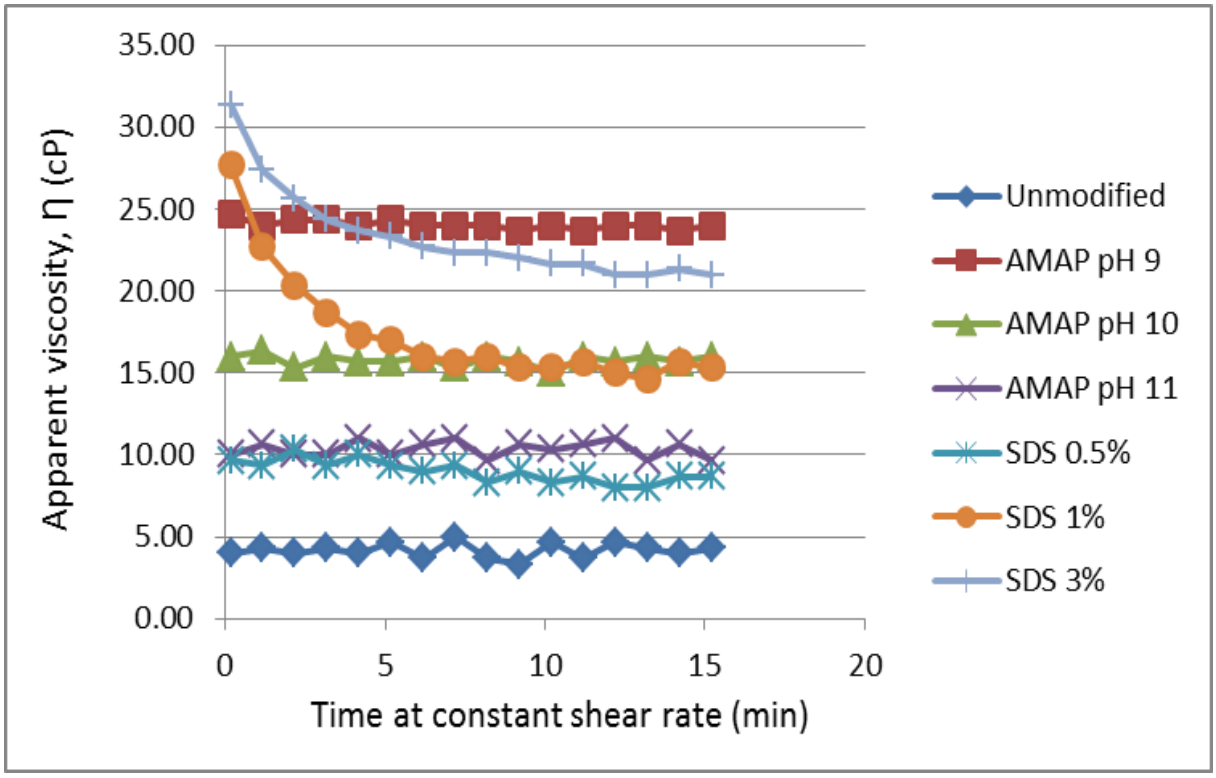

Figure 4.1 Time-dependence of modified algae protein adhesives

4.2.2 Shear rate-dependent behavior

All seven adhesives exhibited non-Newtonian, shear rate-dependent behavior that could be well represented by both the Bingham Plastic equation (2) and Power Law equation (3):

$$\tau = K(\dot{\gamma}) + \tau_o \qquad (2)$$

$$\tau = K(\dot{\gamma})^n \qquad (3)$$

τ = shear stress (D/cm^2)

τ_0 = yield stress (D/cm^2)

$\dot{\gamma}$ = shear rate (s^{-1})

n = flow behavior index

K = consistency index

The values of τ_0, n, K, and the correlation coefficient (R^2) are summarized in Table 4.2. The shear stress of unmodified algae protein adhesive increased linearly with shear rate increase and had a yield stress of 1.64 D/cm^2, making it a Bingham plastic material. The modified algae protein adhesives, however, all displayed shear-thinning behavior (n<1) (Table 4.2 and Figure 4.2).

Table 4.2 Values of power law variables of modified algae protein adhesives

Adhesive formulation	Consistency index K	Flow behavior index n	Yield stress (D/cm^2) τ_0	Correlation coefficient R^2
Unmodified	0.031	1	1.64	0.9887
AMAP pH 9	1.731	0.6072	0	0.9995
AMAP pH 10	0.705	0.6976	0	0.9993
AMAP pH 11	0.337	0.7672	0	0.9951
SDS 0.5%	1.836	0.4506	0	0.9716
SDS 1%	7.334	0.3109	0	0.9831
SDS 3%	2.724	0.5422	0	0.9921

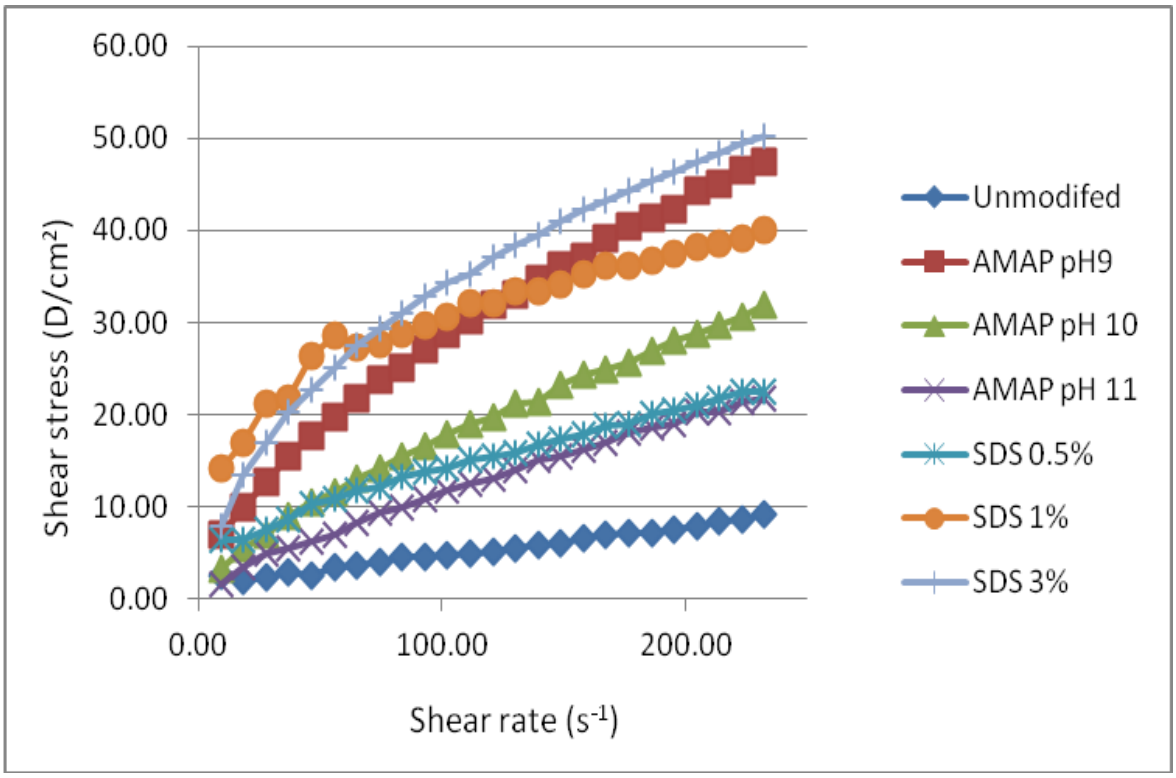

Figure 4.2 Shear rate-dependence of modified algae protein adhesives

4.3.3 Temperature-dependent behavior

The apparent viscosities of all seven adhesives decreased as the temperature increased from 30 to 90°C (Figure 4.3). Chronakis (2001) observed a decrease in viscosity of *Spirulina platensis* protein caused by the increase in kinetic energy as the temperature increased from 30 to 60°C; however, the viscosity of *Spirulina* protein increased between 60 and 80°C due to denaturation. The effect of protein denaturation on the viscosity of the adhesives may not have been as strong due to the relatively lower protein content of the *Cladophora* protein extract (33.43%) compared to the *Spirulina* protein extract (78.6%) used by Chronakis (2001); however, the viscosity decrease does slow after 65°C as the temperature continues to rise.

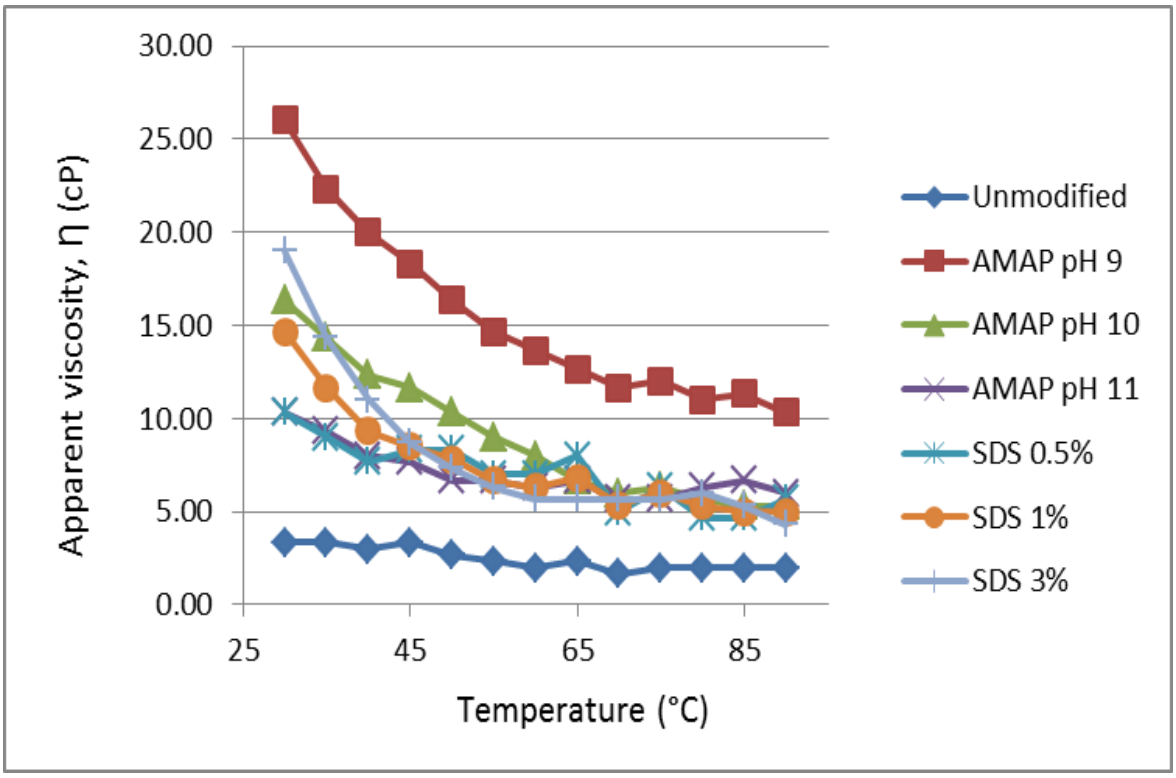

Figure 4.3 Temperature-dependence of modified algae protein adhesives

4.3 Shear strength

Dry, wet, and soaked shear strengths of the modified protein adhesives were measured to assess adhesion performance. Alkali modification increased dry shear strength significantly at pH 9 and 10, but at pH 11 the increase in shear strength was not significant compared to unmodified algae protein (Table 4.3 and Figure 4.4). SDS modification increased dry shear strength significantly at all three concentrations (0.5, 1, 3%). Alkali modification did not significantly improve the water resistance of the adhesives in either the wet or soak shear strength tests. However, SDS modifications of 1% and 3% significantly increased the shear strength of wet wood specimens, and 3% SDS modified algae protein also significantly increased the soak shear strength.

Alkali modification is known to cause unfolding and hydrolysis of soy proteins, which increases surface area and exposes hydrophobic groups, resulting in improved adhesive strength and water resistance of soy protein adhesives (Kumar et al. 2002; Hettiarachchy 1995; Sun and Bian 1999). However, these changes in protein structure are also associated with lower viscosities of both soy protein and *Spirulina platensis* algae protein (Kumar et al. 2002;

Hettiarachchy 1995; Chronakis 2001). Low viscosity has been reported to negatively impact the adhesive performance in soy proteins (Kumar et al. 2002; Lambuth 1997). The decrease in viscosity noted in AMAP pH 11 (Figure 4.2) may be responsible for the observed decrease in shear strength compared to pH 9 and 10.

SDS modification has been reported to improve shear strength and water resistance due to partial denaturation of the soy proteins and creation of ordered, micelle-like clusters (Huang and Sun 2000). SDS-modified algae proteins displayed improved shear strength and water resistance that are consistent with reported results in soy protein (Huang and Sun 2000).

Table 4.3 Shear strengths of wood specimens glued with modified algae protein adhesives*

Adhesive Formulation	Dry strength	Wet strength (MPa)	Soak strength
Unmodified	1.492^{a}	0.146^{e}	0.955^{a}
AMAP pH 9	2.113^{b}	0.217^{ef}	1.432^{a}
AMAP pH 10	2.443^{c}	0.322^{ef}	1.069^{a}
AMAP pH 11	1.921^{ab}	0.294^{ef}	0.874^{af}
SDS 0.5%	1.990^{b}	0.232^{e}	0.675^{af}
SDS 1%	2.875^{d}	0.460^{f}	1.061^{a}
SDS 3%	2.895^{d}	0.466^{f}	2.633^{cd}

*Means, based on n=5, followed by different superscript letters are significantly different using LSD and a probability level of α=0.05.

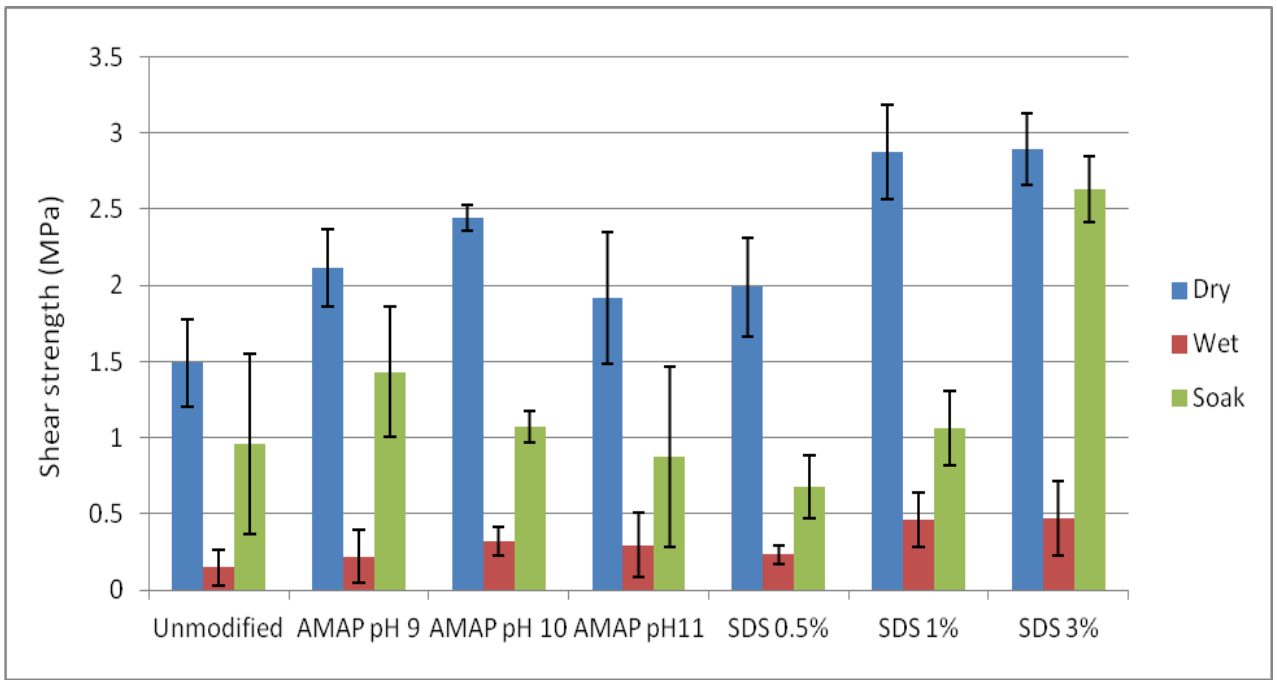

Figure 4.4 Shear strengths of wood specimens glued with modified algae protein adhesives.

4.4 Thermal properties

The glass transition and denaturation temperatures of the modified algae protein adhesives were measured in order to evaluate the changes in protein structure caused by alkali and SDS modification. The algae protein adhesives showed two endothermic peaks, one near 50°C and one near 80°C (Figure 4.5). The peak near 50°C represents the glass transition temperature (T_g) of the protein, while the peak near 80°C is the denaturation temperature (T_d). The observed glass transition and denaturation temperatures of the modified algae protein adhesives are given in Table 4.4. Denaturation enthalpies were not able to be accurately measured due to the broad peaks and the overlap of exotherms caused by decomposition of a non-protein component.

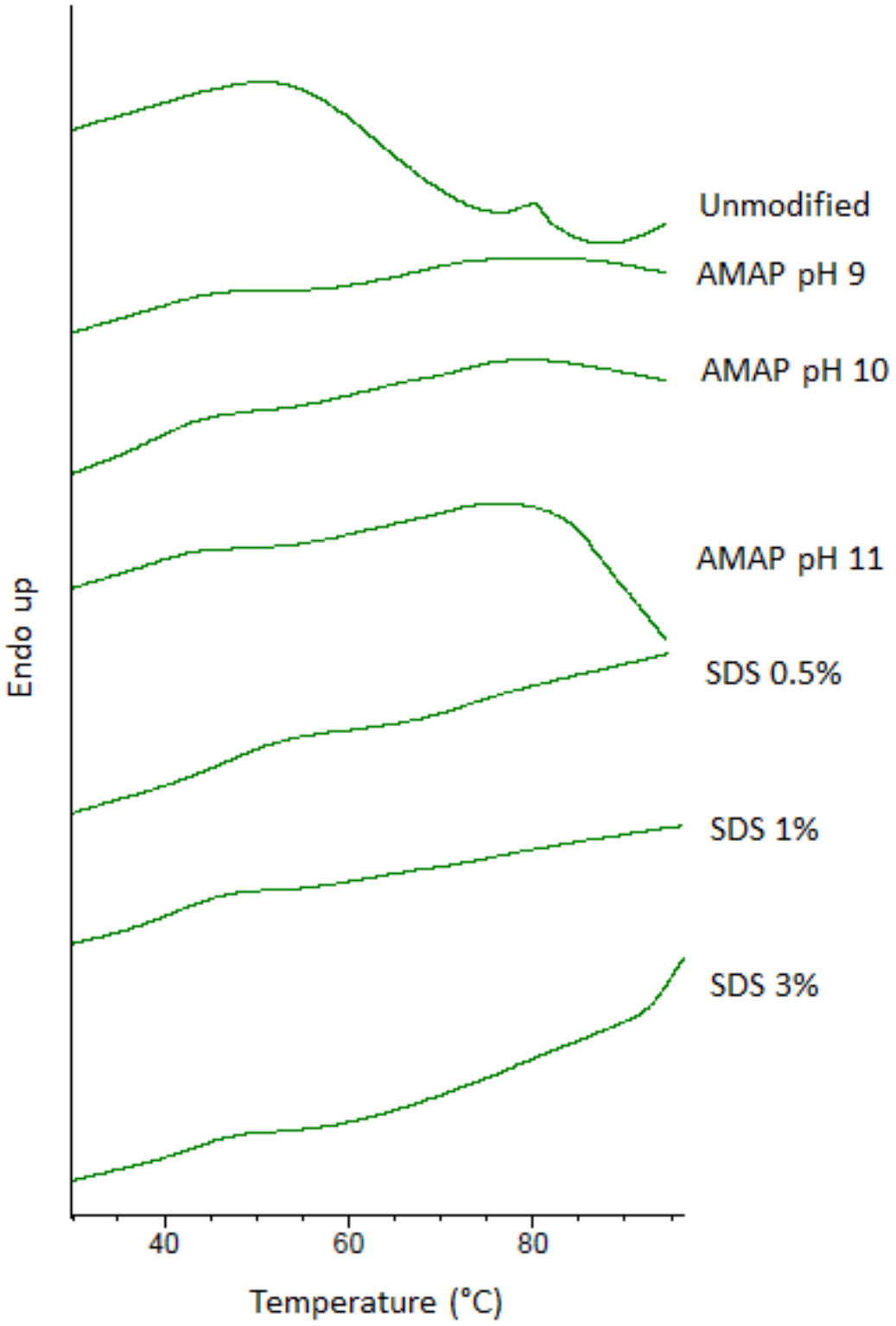

Figure 4.5 DSC thermograms of modified algae protein adhesives

Table 4.4 Glass transition (T_g) and denaturation (T_d) temperatures of modified algae protein adhesives

Adhesive formulation	T_g (°C)	T_d (°C)
Unmodified	54.16	82.44
AMAP pH 9	48.33	82.57
AMAP pH 10	46.97	81.27
AMAP pH 11	46.34	80.13
SDS 0.5%	55.29	-
SDS 1%	49.38	-
SDS 3%	48.06	-

The T_g values of alkali-modified proteins were lower than unmodified and decreased as pH increased from 9 to 11. Similarly, the T_d values of alkali-modified proteins decreased with increasing pH, although the T_g of AMAP pH 9 was approximately the same as that of unmodified algae protein. The decrease in T_g at pH 9 indicates a less ordered protein structure, likely caused by decreased particle aggregation, even though no significant denaturation has taken place (Chronakis 2001). As the pH is further increased, the algae proteins begin unfolding and this denaturation leads to lower T_d values. Chronakis (2001) reported similar decreases in T_d at basic pHs for *Spirulina platensis* protein.

The T_g of 0.5% SDS-modified algae protein was slightly higher than that of unmodified algae protein. The higher T_g value indicates a more crystalline, brittle structure in the 0.5% SDS-modified proteins, which is consistent with the ability of SDS to form more ordered structures by disrupting hydrophobic and electrostatic bonds and binding to the protein chain (Huang and Sun 2000; Mo and Sun 2000). The decrease in the glass transition as SDS concentration increased could be due to the further breakdown of protein-protein interactions that could not be compensated for by the SDS-protein interactions. No clear endothermic denaturation peaks were observed for the SDS-modified algae protein. This indicates that the protein was completely denatured during SDS modification. The relatively low protein concentration in the adhesives could be responsible for the high level of denaturation even at low SDS concentrations.

The higher level of denaturation of SDS-modified algae protein compared to alkali-modified and unmodified proteins could be responsible for the higher shear strengths and improved water resistance of SDS-modified algae proteins (Table 4.3). More unfolded proteins

could increase protein-protein and protein-wood interactions to increase the shear strength. The increased viscosity of SDS-modified algae proteins provided supporting evidence for the unfolding and denaturation of the algae proteins (Figure 4.1) and also is known to improve shear strength (Kumar et al. 2002).

4.5 Morphology

The effects of alkali and SDS-modification on the particle size and surface texture of algae proteins were examined using scanning electron microscopy (SEM) (Figure 4.6). The unmodified algae protein formed irregular plates or disks, with a few visible cylindrical particles (A). Alkali modification progressively decreased the overall particle sizes and resulted in more sharp and jagged edged particles (B-E). SDS modification also decreased particle size (F-G).

At higher magnification, surface microstructure of the modified algae proteins, which plays an important role in adhesion, was visible. The surface of unmodified algae protein was course with small dimples and peaks visible (Fig. 4.6 a). Alkali modification smoothed the surface of the particles considerably, possibly due to the hydrolysis of exposed peptides (b-e). Conversely, SDS modification created much rougher surfaces (f-g). At 0.5% and 1% SDS modification, small ovoid and cuboid structures were visible on the surface (e-f), while at 3% SDS modification, small holes and fibrous linkages are visible (g). The respective smoothening and roughening effects of alkali- and SDS-modification may represent two different mechanisms for improving adhesion. The smooth alkali-modified protein would have a larger surface area in direct contact with the wood substrate to increase electrostatic interactions, while the rougher surface of the SDS-modified protein would promote mechanical interactions with the peaks and grooves of the wood substrate (Kumar et al 2002).

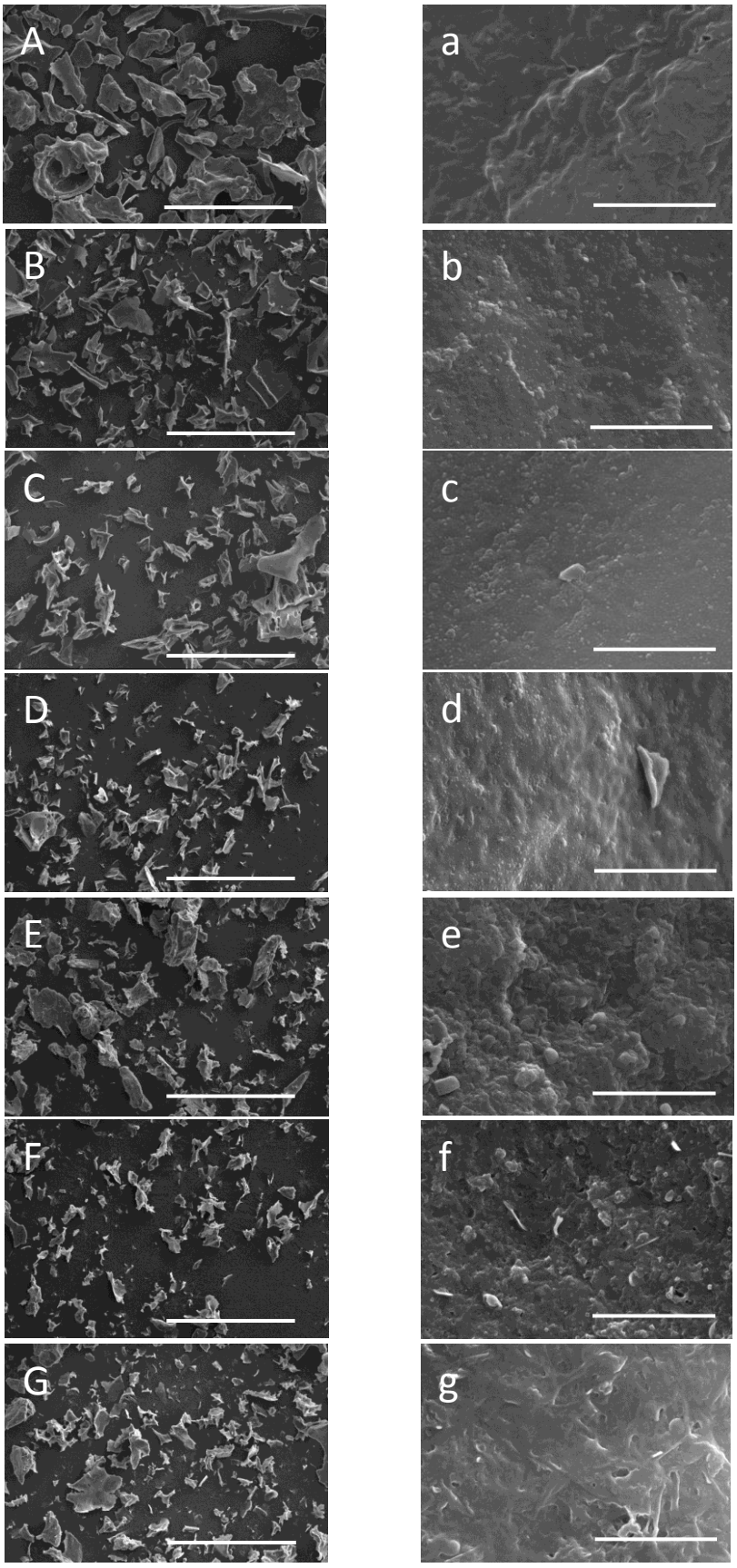

Figure 4.6 SEM micrographs of modified algae protein.
(A) a – Unmodified; (B) b – AMAP pH9; (C) c – AMAP pH 10; (D) d – AMAP pH 11; (E) e – SDS 0.5%; (F) f – SDS 1%; (G) g – SDS 3%. Magnification: (A)-(G), x100; a-g x10k. Horizontal bars represent 500 and 5 µm for x100 and x10k magnifications, respectively.

Chapter 5 - Conclusions and Recommendations

Algae biomass has been researched extensively as an alternative source of fuels and co-products; however, little research has focused on the use of algae protein. Utilizing algae protein to develop value-added products as part of a biorefinery strategy has the potential to increase the economic feasibility of algae biodiesel. This research explored the use of algae protein extracted from *Cladophora* sp. and modified with sodium hydroxide and sodium dodecyl sulfate for adhesives. Mechanical properties of algae protein adhesives with different modifications were studied along with morphological, rheological, and thermal properties of the modified algae protein adhesives.

Rheological studies indicated that alkali modification resulted in an initial increase in apparent viscosity compared to unmodified algae protein due to increased solubility, followed by a decrease in viscosity as the proteins were partially denatured. On the other hand, the viscosity of SDS-modified algae proteins increased as SDS concentration increased. This was caused by protein swelling, denaturation, and formation of ordered SDS-protein structures. Thermal analysis verified the complete denaturation of algae proteins by SDS modification and partial denaturation by alkali modification. Moreover, SEM analysis revealed the morphological changes caused by modification. Alkali- and SDS-modified algae proteins both displayed decreased particle sizes compared to unmodified algae proteins; however, alkali-modification smoothed the surface of the particles while SDS modification resulted in rougher surfaces. All of these properties were reflected in the observed differences in shear strength of the adhesives. Although both alkali- and SDS-modified algae protein increased the dry shear strength compared to unmodified algae protein, only 3% SDS-modified algae protein was able to significantly improve the water resistance as measured by wet and soak shear strength tests. The complete denaturation, rough surface, and high viscosity of SDS-modified algae protein resulted in the increased shear strength and water resistance.

Although the shear strengths of all the modified algae protein adhesives were lower than reported values for similarly modified soy protein adhesives, further research has the potential to increase the adhesion performance to a level that is competitive with soy protein adhesives. Recommendations for future research include the following:

1. Improving protein extraction and isolation so that the protein purity is comparable to that of soy protein isolate.
2. Identifying algae species with both protein and lipid compositions suitable for adhesive and biodiesel production.
3. Exploring other adhesive formulations and protein modifications, such as varying protein concentration, chemical modifications, and blends.
4. Performing shear strength studies on a variety of wood types as well as pressing conditions.

This study serves as a crucial first exploration of the use of algae proteins for adhesive development. The feasibility of improving the shear strength and water resistance of algae protein adhesives through modifications commonly applied to soy proteins has been proven. Further research holds the potential of making modified algae protein adhesives a viable and environmentally-friendly alternative to both petroleum-derived and soy protein adhesives.

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