

BIO-PLASTIC POTENTIAL OF SPIRULINA MICROALGAE

by

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(Under the Direction of Suraj Sharma)

ABSTRACT

Spirulina (SP) alga biomass was used to make bio-plastics using compression molding. Plasticization using ethylene glycol (EG), blending with ultra-high molecular weight polyethylene (UHMW-PE), and compatibilization using polyethylene-graft-maleic anhydride (PE-g-MA) with the Spirulina biomass of proper composition were tried to develop better performance bio-plastics than those made of 100% Spirulina biomass. Activated carbon, as a scavenger, can effectively absorb unpleasant odors from algae bio-plastics. Proteins were extracted from Spirulina. The protein content was determined by Bicinchoninic Acid (BCA) test assay, and Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to obtain the approximate molecular weight. Spirulina and its extracted protein show good potentials of developing bio-plastics.

INDEX WORDS: Bio-plastic, Spirulina, Microalgae, Protein

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CHAPTER 1

INTRODUCTION

Background

While traditional petroleum-based plastics made of synthetic polymers are employed in many ways, they charge a high price for the large amount of energy they consume during production and their resistance to degradation. Conventional plastics harm the environment since 1) they are made of crude oil which is a diminishing natural resource; 2) they do not undergo bacterial decomposition so that landfills only preserve them for centuries, and 3) their incineration releases poisonous chemicals.

One way to resolve the environmental problems is to recycle the plastics. However, this process faces many challenges. Much heat and energy are required to recover the waste plastic. That is because plastics have low entropy of mixing due to the high molecular weight of their large polymer chains. The separation of waste plastic material into different single polymers, though time consuming, is usually necessary. If different types of plastics are melted together, they tend to phase-separate and the phase boundaries would cause structural weakness in the resulting material. Also, dyes, fillers, and other additives in plastics are difficult to remove [1]. Another solution lies in the development of bio-plastics which are at least partially biodegradable and less petroleum dependent [2] and are promising alternatives to conventional plastics.

Purpose of This Study

This study is to explore different ways to enhance the properties of bio-plastic made from Spirulina and to find a better and more reliable approach for making Spirulina based bio-plastic. Spirulina biomass can be processed into bio-plastics by means of plasticization, blending and compatibilization. Among the components of Spirulina, protein is of importance in developing Spirulina biomass into polymer. Using extracted Spirulina protein was an alternative way to evaluate the bio-plastic making potentials of Spirulina. Thermal, mechanical, and morphological properties of the above mentioned bio-plastics were measured to assess their performances. Activated carbon was used to study its effectiveness in odor removing of the Spirulina bio-plastic.

This research was an exploration of the potential of algae-based bio-plastic as an alternative to replace conventional plastics. The reasons why algae serve as a good candidate for making bio-plastics are: first, algae based bio-plastic was less petroleum dependent and more degradable, second, the procedure is straight-forward and cost effective, last, compared to other biomass, algae are of high biomass yield, low-cost, and simplicity of cultivation, with little impact on the food chain. Therefore, developing an algae based bio-plastic is a good solution to solving some of the problems caused by the wide use of conventional plastics.

Definition of Terms

Bio-plastic

Bio-plastics are materials that contain biopolymers in various percentages and can be molded by heat action and pressure. According to ASTM D6866-06, biopolymers involve living organisms in their synthesis processes and therefore have partial or total biochemical origins from natural, renewable materials and can be biodegradable [3]. In general, three approaches are

taken to produce biopolymers: polymers extracted directly from biomass either with or without modification; polymers produced with renewable raw materials and obtained by means of bio-intermediaries; and polymers produced directly by microorganisms in their natural or genetically modified state. Figure 1.1 shows the common biopolymers developed [4][5].

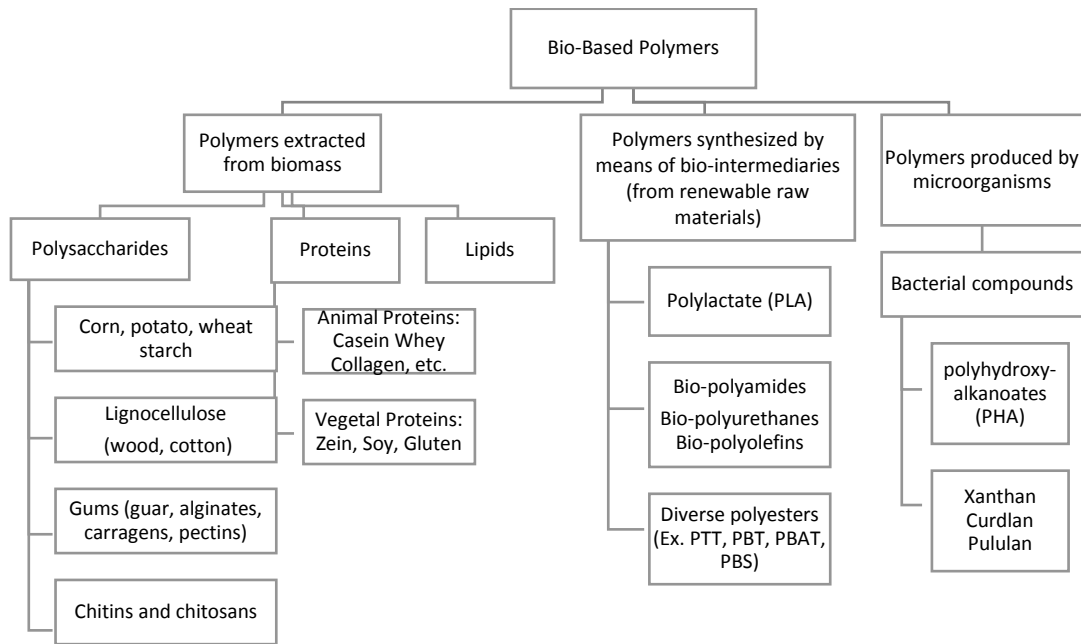


Figure 1.1 Some Common Types of Biopolymers

In this context, algae based bio-plastics belongs to the first group which is often also called agro-polymers. The agro-polymers can be processed directly into thermoplastic materials, but most require chemical modification. They have some common characteristics such as hydrophilicity, fast degradation rates and sometimes unsatisfactory mechanical properties, particularly in wet environments [6]. Therefore, the main technological challenge is to successfully modify the properties of these materials to overcome deficiencies such as brittleness, water sensitivity and low strength.

Algae and Spirulina

Algae are photosynthetic organisms that occur in most habitats, ranging from marine, to fresh water to desert sands and from hot boiling springs to snow and ice. They vary from small, single-celled forms to complex multicellular ones [7]. Microalgae are unicellular species which exist individually, or in chains or groups typically found in freshwater and marine systems. They are capable of performing photosynthesis but do not have roots. Macroalgae are multicellular and plant-like algae which have roots. Some common groups of algae are Chlorophyta (the green algae), Rhodophyta (the red algae), Phaeophyta (the brown algae), Cyanophyta (the blue-green algae), etc. among which the former three are macroalgae while the last one belongs to microalgae.

Algae are of significant importance to the environment. Firstly, algae have high photosynthetic efficiencies and are important as primary producers of organic matter at the base of the food chain and provide oxygen for other aquatic life. Secondly, algae can be produced in many harsh environments not suitable for crop production, including non-arable land, saline and wastewater, and they can grow very fast with a short life cycle.

Algae can also be good sources to produce bio-plastics. Among all the approaches to make bio-plastics, deriving bio-plastics directly from biomasses is the most straight-forward and cost effective way. For the commonly used biomasses, such as corn starch and soybean protein, a large amount of resources like the farm land, water and fertilizers, and time and energy are required. Aside from the terrestrial crops, another good option may be algae which also contain components such as protein and carbohydrates that are crucial for developing bio-plastics. In fact, compared to all other sources, algae are the best choice for bio-plastic production due to their high biomass yield, low-cost, simplicity of cultivation, and little impact on the food chain.

Spirulina platensis is a microalga, or more specifically, cyanobacteria, typically found in high-alkaline freshwater conditions. It is widely used in the nutraceuticals industry. *Spirulina* is rich in protein. Dried *Spirulina* contains about 60% (51–71%) protein. Its protein contains all essential amino acids [8]. *Spirulina* was used in this research.

Plasticizer and Plasticization

Plasticizers are generally small, relatively non-volatile, organic molecules that are added to polymers to improve flexibility (by reducing brittleness), durability (by increasing toughness and reducing crystallinity), and processability (by lowering glass transition and melting temperatures). There are two types of plasticizers, internal and external plasticizers. The internal plasticizers would become part of the polymer molecules by being either copolymerized or grafted to the polymer structure. This results in difficulty for the polymer chains to compact closely. The external plasticizers that are nonvolatile would swell and greatly influence the intermolecular forces within the polymer plasticizer system.

The plasticization reduces the relative number of polymer-polymer contacts thereby decreasing the rigidity of the three dimensional structure, allowing deformation without rupture. Three theories are explored to interpret the plasticization mechanism. Lubricity theory regards the plasticizer as a lubricant that reduces friction and facilitates mobility between polymer chains. The gel theory suggests that plasticizer disrupts and replaces intermolecular forces between polymer-polymer chains to form a polymer gel structure with increased flexibility. The free volume theory proposes that plasticizers increase and maintain the free volume of resins during processing and cooling phases and lead to lower glass transition temperature [9].

When plasticizers are compared based on the mass fraction in a bio-plastic, low molecular mass compounds such as water was present in larger numbers compared to high

molecular mass compounds. Every plasticizer molecule can interact with a protein chain, which implies that at equal mass fractions, water is normally more efficient than other plasticizers [10]. However, the low boiling temperature of water is a serious complication during processing as evaporation typically occurs before adequate processing temperatures for low enough viscosities are reached [11]. Alternatively, hydrophilic compounds such as polyols, carbohydrates and amines may also interact with the polar groups in agro-polymers, thereby plasticizing the material. Some examples are glycerol, sorbitol, saccharose, urea, triethylene glycol and polyethylene glycol [10]. These molecules must be polar to ensure compatibility with the polymers and small enough to penetrate the macro-molecular network.

Blending

Blending is a commonly used method to modify the properties of a polymer. Blending involves a physical mixing of multiple polymers. The resulting polymer would exhibit the properties of all the polymers comprising the mixture if a relatively uniform phase is achieved. The compatibility of the polymers determines the structure and properties of the resulting polymer blend. The polymers involved should be thermally compatible to avoid two or more phases occurring. If the polymers are compatible, there should be only one phase and therefore only one glass transition temperature that lies between the glass transition points of the respective polymers. If the polymers are partially compatible, the phase was not exhibit a continuous form and two approaching glass transition points are expected. If the polymers are not compatible, two phases and two glass transition points representing two polymers will be present.

Compatibilizer and Compatibilization

The major disadvantage of incorporating a natural polymer into a synthetic polymer is their compatibility. Natural polymers are hydrophilic whereas synthetic polymers are hydrophobic in nature. The resultant blend of these two types of polymers is generally immiscible [12]. Compatibilizers, also referred to as coupling agents, are additives. These modify the interfacial properties and stabilize the melt blend. One of the most successful techniques for compatibilization is the use of thermoplastic maleic anhydride graft copolymers [13]. The thermoplastic part of the compatibilizer is favored by the synthetic component of the blend while the maleic anhydride part is favored by the natural polymer. Therefore, compatibilizer stabilizes the blend resin.

Literature Review

Though many experiments have been done to generate bio-plastics using soy protein, corn starch, etc., not much work has been done to develop bio-plastics using algae. Otsuki, T. et al. (2004) developed a composite of the green microalga *Chlorella* sp. with polyethylene (PE) through chemical modification of PE with maleic anhydride (MA) and shaped it into plate and dish-like moldings by a heat-pressurizing method. They found that the interaction of *Chlorella* grains with a PE matrix was strikingly enhanced by the chemical modification of PE with MA. Compared to unmodified PE, the *Chlorella*-MA modified PE composite (Ch-MPE) had a great increase in tensile strength using a 40 wt % content of *Chlorella*. This marked increase was attributed to the formation of chemical bonds between *Chlorella* grains and the PE matrix confirmed IR and SEM studies [14]. In Chiellini, E. et al. (2008), the green alga *Ulva* were positively evaluated for the production of composites with poly(vinyl alcohol) (PVA) polymer (which is hydrophilic, and eco-compatible) as the continuous matrix by casting of aqueous

suspensions and compression molding. Plasticization using glycerol enhanced the processing of PVA, Ulva, and starch blends. The positive results of film-forming properties and mechanical characteristics of blends even with limited amounts of PVA (40%) attested to Ulva's suitability to be introduced in composites (up to 30%). [15]. Zeller, M.A. et. al. (2012) evaluated duckweed's potential for plastic production through compression molding and investigated the stability and thermal characteristics of plasticized and blended duckweed polymers. They found that a 3: 1 ratio of duckweed to glycerol produced the best polymer stability. The blends of plasticized algae biomass (with 3:1 weight ratio of duckweed to glycerol) with polyethylene (PE) demonstrated dispersion in biobased or polyethylene (PE) phase, except for 50/50 biobased/PE where phase continuity was observed. Also, the surface morphology indicated limited homogeneity in blends and increased PE was correlated with increased temperature stability of the biobased phase [16].

Algae-based bio-plastics belong to agro-polymers which are a kind of bio-polymers processed directly from extracted biomass either with or without modification. Processing polymers means mixing and shaping of polymeric materials to form them into useful products. The process usually involves the application of heat and pressure. Pressure molding to make thermoplastics is adopted in this research. That is because by heating hydrophilic polymers (such as protein) in closed volumes in the presence of plasticizer, homogeneous melts may be formed which can be processed like conventional petrochemical-based thermoplastics [17]. The transformation of agro-polymers into a thermoplastic is often more complex than conventional thermoplastic processing. The diversity of polymer structures and interactions, as well as the dependence of their structure on the extraction techniques followed complicate processing. Furthermore, hydrogen bonding is strongly water-sensitive which makes processing this class of

polymers even more complex [11]. Physiochemical properties and processing conditions are often governed by the protein's structural properties, and therefore also the final material properties [18].

The most important component of algae that contributes to formation of polymers is the proteins. This is different from synthetic polymers. A synthetic polymer consists of identical monomers, covalently bonded in a long chain. Unlike synthetic polymers, proteins are complex hetero-polymers, consisting of up to 20 different amino acids. The amino acids each contain two carbon atoms as well as nitrogen, differing only in their functional side groups. In its natural environment, a protein was folded into secondary, tertiary and quaternary structures stabilized through hydrophobic interactions, hydrogen bonding and electrostatic interactions between amino acid functional groups. Proteins are hetero-polymers with combinations of hydrophobic, hydrophilic, acidic and basic side chains and have a wide range of different intermolecular interactions compared to synthetic homo-polymers. The folded conformation is a delicate balance of these interactions [19]. Once folded, the structure may be stabilized further with strong covalent crosslinks. Due to the diverse building blocks of proteins and their unique structures, a large variety of biodegradable materials can be produced offering a wide range of functional properties [10]. Polymer chains are typically linked by a multitude of interactions such as hydrogen bonding, hydrophobic interactions and other weak van der Waal's forces. Inter- and intra-molecular bonds, as well as chain entanglements, tend to prevent chain slippage, thus leading to the superior properties of polymers.

Generally speaking, agro-polymers have relatively low degradation temperatures and the energy required to disrupt intermolecular bonding is close to the energy leading to degradation [11]. Most agro-polymers do not behave thermo-plastically without some additives and would

typically degrade before a flowable melt can be formed and thus some requirements need to be fulfilled to make bio-plastics.

Three broadly categorized processing requirements have been identified for protein-based thermoplastic processing [10]: breaking of intermolecular bonds (non-covalent and covalent) that stabilize proteins in their native form by using chemical or physical means; arranging and orientating mobile chains in the desired shape; enabling formation of new intermolecular bonds and interactions to stabilize the three-dimensional structure. To break intermolecular non-covalent bonds, denaturation is needed. Denaturing of protein occurs when small changes in environmental conditions, such as increasing temperature, pressure, change of pH or addition of chemicals can disrupt a protein's folded conformation. Denaturation is a unique property of proteins and can be defined as the modification of secondary, tertiary or quaternary structures of a protein molecule. To arrange mobile chains in the desired shape, heat and pressure are applied. To enable formation of new intermolecular bonds and interactions to stabilize the three-dimensional structure, plasticizers are added. These help reduce the intermolecular interactions between the agro-polymer chains. Also, blending of the algae biomass with synthetic polymers and compatibilizers that help hold the two components together are used. Proteinaceous bio-plastics are often brittle and water sensitive, and overcoming this is one of the driving forces behind research in this field.

Contents Studied in This Research

Firstly, several kinds of algae were investigated for their potentials for bio-plastic production. Secondly, the performances of the Spirulina algae biomass, and those of its modified ones such as plasticized by Ethylene Glycol, blended with ultra-high molecular weight polyethylene (UHMW-PE) were tested to explore suitable formulations that could be used for

making Spirulina bio-plastics. Thirdly, the effectiveness of compatibilizer was evaluated by an addition of polyethylene-graft-maleic anhydride (PE-g-MA) with 3% weight ratio to the UHMW-PE blended Spirulina bio-plastic. Also, the activated carbon as a scavenger was used for eliminating the uncomfortable odor generated from Spirulina plastics flexbar. Last, the protein was extracted from Spirulina. The protein content was confirmed by using BCA assay and the protein molecular weight was informed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The extracted proteins were used to make bio-plastics.

Methodology

In this study, empirical methodology was used since data was collected; experimental methodology was used since variables are going to be manipulated; quantitative methodology was used.

Proximate Composition Analysis

The proximate composition analysis was performed on a LECO TGA701 by a standard protocol. This test method produces data showing moisture, volatiles, ash, and fixed carbon percentages consecutively. To get the crude protein content, elemental analysis was conducted using an LECO (Model CHNS-932, LECO, St. Joseph, MI) analyzer following methods outlined in ASTM D 5291 and D 3176. The crude protein content is estimated by multiplying the elemental N content by a factor of 6.25 [20]. For all the samples, triplicates were run to get the average results.

Bio-plastics Processing

To prepare the algae bio-plastic, thermo-mechanical molding of the samples was performed using a 24-ton bench-top press (Carver Model 3850, Wabash, IN) with electrically

heated and water-cooled platens. The stainless steel molds can form either a single dogbone for Instron analysis or two rectangular flexbars for DMA analysis at one time. Each different weight ratio formulation was thoroughly hand mixed. Compression molding of samples used a 20-min cook time at 150°C followed by a 10-min cooling period, and both were performed under pressure larger than 24,000Pa [16].

Thermal Properties Analysis

Thermal analysis can provide the information about the changes of the samples through the heating process. Through thermal gravimetric analysis (TGA), the changes in physical and chemical properties of materials are measured as a function of increasing temperature (with constant heating rate). Differential scanning calorimetry (DSC) reveals the difference in the amount of heat required in increasing the temperature of a sample and reference.

TGA was performed using a Mettler Toledo TGA/SDTA851e and DSC was performed using a Mettler Toledo DSC821e. TGA was performed from 25-500°C under N₂ gas with a heating rate of 10°C/min. DSC was performed from -50 to 250°C under N₂ gas with a heating rate of 20°C /min. All samples were prepared with sample weights between 4 and 10 mg [16]. For the plastic samples, fine pieces were cut from DMA flexbars before running TGA and DSC.

Mechanical Properties Analysis

Dynamic mechanical analysis (DMA) and tensile testing were used to characterize the mechanical properties of the bio-plastics. DMA is useful in studying the viscoelastic behavior of polymers. It evaluates the elastic modulus G' (or storage modulus), which is related to the stiffness of the material, and the viscous modulus G'' (or Tan Delta), which is related to the potential of energy absorption in the sample. It can be used to locate the glass transition

temperature of the material, as well as to identify transitions corresponding to other molecular motions. Dynamic mechanical analysis (DMA) was performed on a DMA8000 Dynamic Mechanical Analyzer from Perkin Elmer for specimens with dimensions of 9 (width) \times 2.5 (thickness) \times 12.5 (length) mm using a dual-cantilever setup at a frequency of 1 Hz. All samples was run with a displacement of 0.05 mm from room temperature to 160°C at a temperature ramp of 2°C/min. All samples were run in duplicate to ensure reproducibility [16].

Tensile properties such as stress and extension at maximum load were measured using the Instron testing system (Model 3343) interfaced with a computer operating Blue Hill software. The test was performed under a controlled environment (20°C, 65% RH), according to the standard test method for tensile properties of plastics (ASTM D638-86) at 5 mm min⁻¹ crosshead speed with a static load cell of 1000 N and gauge length of 60 mm. The specimens were conditioned at standard conditions (20°C, 65% RH) for 24 h before testing and run in duplicate [16].

Morphology Observations: Scanning Electron Microscopy (SEM)

For the morphology observations, Scanning electron microscopy (SEM) technique was used. To prepare plastic samples for SEM observation, the DMA flexbar was submerged into liquid nitrogen for 20s which breaks the sample immediately and fracture surfaces was observed after 60 seconds of gold coating. The best formulation can be determined through observation of the fracture surface of the bio-plastic samples since better material phase continuity means a better plasticization effect of Ethylene Glycol. SEM images were recorded on a Zeiss 1450EP variable pressure scanning electron microscope [16].

Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. The plasticization and crosslinking would make some change in the absorption bands of the amide groups and H-bonding that can be observed by FT-IR. Important changes after thermoplastic processing investigated by FT-IR included changes in secondary structures (α -helices and β -sheets or turns) of proteins and the interaction between chains in proteins and plasticizers.

Gas Chromatography–Mass Spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography (GC) and mass spectrometry (MS) to identify different substances within a test sample. The GC can separate and analyze compounds that can be vaporized without decomposition, while the MS produces spectra of the masses of the atoms or molecules comprising a sample of material which helps to identify the material. The GC-MS can help to separate and detect the volatile odor substances in the Spirulina biomass based bio-plastic and to evaluate whether activated carbon effective in odor removing or not.

Protein Extraction

For extraction of algae protein, Fleurence et al. [21] tried various extraction procedures such as extraction using deionized water, Tris-HCl buffer, aqueous polymer two-phase system (PEG/K₂CO₃), and polysaccharidases. They found that a first extraction with deionized water, and a second extraction with NaOH (0.1M) yielded the highest amount of extracted protein. To precipitate protein from the extraction supernatant, trichloroacetic acid (TCA) was used. This is

because some substances such as phenol, or glucosamine was affect protein analysis (both Lowry and Bradford method) since they was either increase the absorbance or decrease the measurements by inhibiting the action of specific reagents, their influence may be avoided by precipitation of the protein sample with TCA [22]. 0.18-0.34M TCA is effective in precipitating only protein, so that small peptides and free amino acids do not affect the protein analysis [23]. Also, the use of a blender instead of incubation is better to extract protein [22].

Bicinchoninic Acid (BCA) Protein Assays

In general, there are two types of protein tests. The first type is based on protein-copper chelation and the secondary detection of the reduced copper. For example, the biuret reaction, bicinchoninic acid (BCA) protein assays and the Lowry protein assays. Another type is based on protein-dye binding with direct detection of the color change associated with the bound dye. Examples are coomassie dye (Bradford) protein assays and Pierce 660nm protein assay. The advantages of the BCA method are that it is straight forward and sensitive to protein concentration and of good linearity. The mechanism of the BCA protein assay is a basically two- step-reaction. In the first step, peptides containing three or more amino acid residues form a colored chelate complex with cupric ions (Cu^{2+}) in an alkaline environment containing sodium potassium tartrate. Because polypeptides have a structure similar to biuret, they are able to complex with copper by the biuret reaction. By reducing the copper ion from cupric to cuprous form, the reaction produces a faint blue-violet color. Single amino acids and dipeptides do not give the biuret reaction, but tripeptides and larger polypeptides or proteins was react to produce the light blue to violet complex that absorbs light at 540nm. In the second step, two molecules of BCA bind to each molecule of copper that had been reduced by a peptide-mediated biuret reaction. Chelation of BCA with the cuprous ion, results in an intense purple color. The

BCA/copper complex is water-soluble and exhibits a strong linear 562nm absorbance with increasing protein concentrations [24].

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Polyacrylamide gel electrophoresis (PAGE) is widely used to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. For proteins, sodium dodecyl sulfate (SDS) is an anionic detergent applied to protein samples to denature the protein into helical structures and also impart a negative charge to complexes with proteins. Since the mass-to-charge ratios for most proteins are similar, the mobilities of proteins going through the gel depend mostly on their size, hence their molecular weights. The smaller protein molecules will migrate faster than the bigger ones [25]. Therefore, several bands of proteins of different molecular weight will form through the gel electrophoresis and would be visualized by the dye stain.

CHAPTER 2

BIO-PLASTIC OF ALGAE BIOMASS

Materials and Experiments

The algae materials include both microalgae and macro algae—the seaweeds, which are multicellular and plant-like algae which have roots. Microalgae are unicellular species which exist individually, or in chains or groups typically found in freshwater and marine systems. They are capable of performing photosynthesis but do not have roots.

The three microalgae samples were received from ALGIX, LLC. These were supplied by the companies of Solix, GO2 and AlgaEvolve, respectively. Solix Microalgae is a nannochloropsis species which are mostly found in the marine environments but also occur in fresh and brackish water [26]. GO2 and AlgaEvolve are green algae consortiums which are mixed species cultures. They were all ground to powder form.

Except for the Ireland Seaweed (green algae) which were provided by ALGIX, LLC, the other three different species of macro were provided by University of Puerto Rico in dry and short stalk status, including Ulva (green algae) and Fucus (brown algae) both from Cape Cod, Massachusetts; and Gracilaria (red algae) from Waquoit Bay, Massachusetts. The samples were then milled to particles which had at least one dimension less than 850 μm .

There are also two algae-based polymer samples, PP-algae and PBAT-algae, provided by ALGIX, LLC. Both of the polymers incorporated jet milled chlorella (a species of microalgae). They were produced by extrusion/compounding and chopped into short cylinders (or pellets). PP-Algae comprised 45% algae, 50% polypropylene (PP) base resins and 5% PP-Acrylic Acid as

compatibilizer. PBAT-Algae comprised 50% Algae and 50% poly(butylene adipate-co-terephthalate (PBAT) . Table 1 shows the types of the received algae materials:

Table 1. Types of the Received Algae Materials

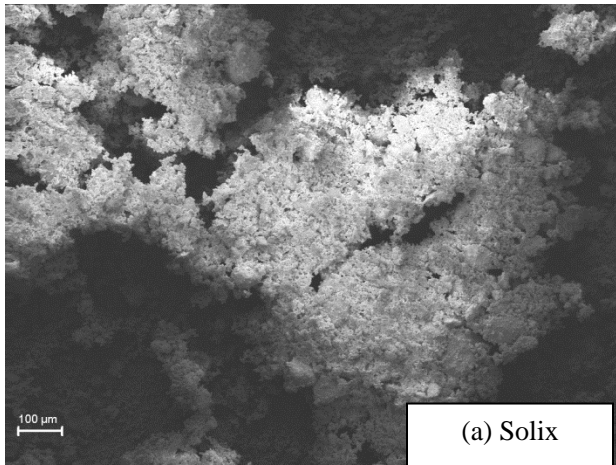
Algae Raw	Micro Algae	Solix Micro, GO2 Algae, AlgaEvolve
Material	Macro Algae	Ireland Seaweed, Ulva, Fucus, Gracilaria (Waquoit Bay),
Algae based Polymer		PP-Algae, PBAT-Algae

The algae and algae-based polymer samples were observed using scanning electron microscopy. Algae plastics were processed using thermal mechanical molding. Thermal properties were tested using thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). Dynamic mechanical analysis (DMA) was used to test the mechanical properties of the bio-plastics.

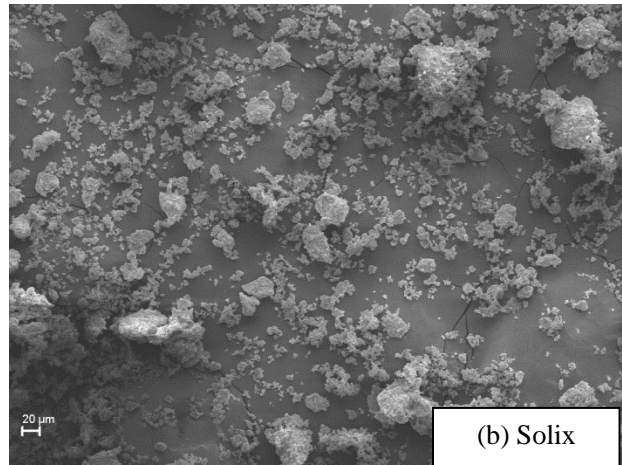
Results and Discussion

Morphology of Algae Biomass

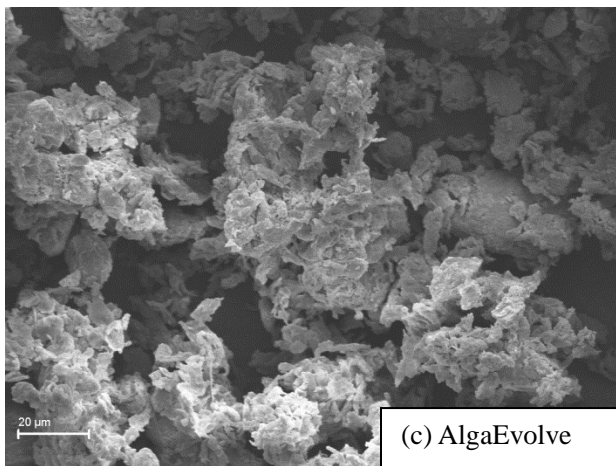
The Scanning Electron Microscopy images of Algae biomass samples are shown in Figure 2.1. These images of the microalgae show no specific features except for the randomly stacked and distributed piles of cells. This may be because the microalgae samples were raffinated and the structures of the cells were ruptured and destroyed. Also, the microalgae were too small to distinguish their morphological characteristics. However, the images of the macroalgae show orderly arranged cavities with some structured cells growing inside. The features of the cells may be distinguishable. The images of the algae-based polymer samples show generally homogeneous distribution of the polymers suggesting the algae’s potential of making thermoplastic blends with traditional resins to make them more environmentally friendly.



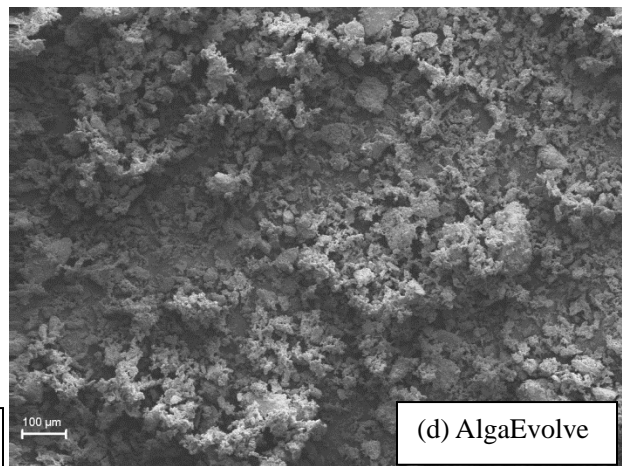
(a) Solix



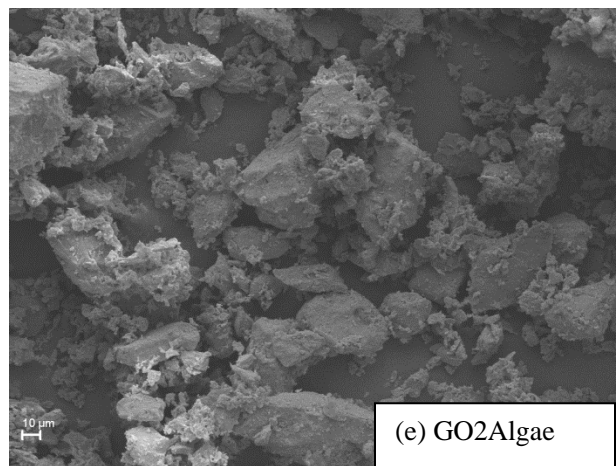
(b) Solix



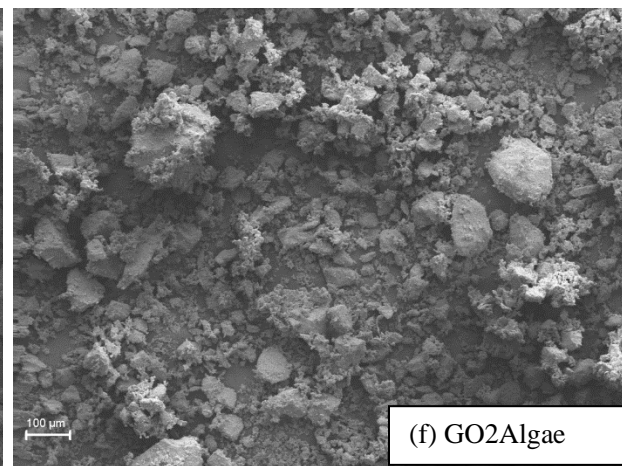
(c) AlgaEvolve



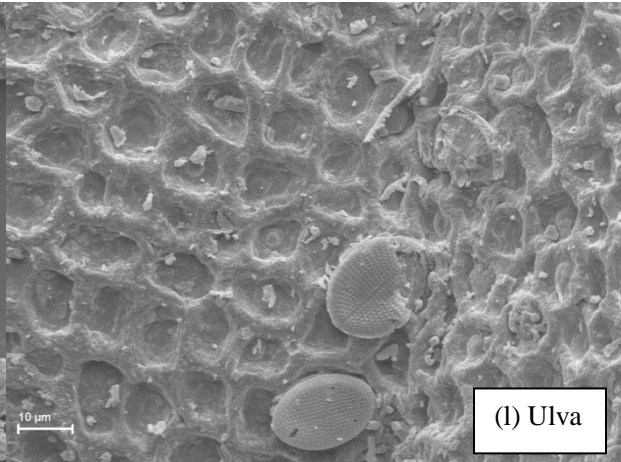
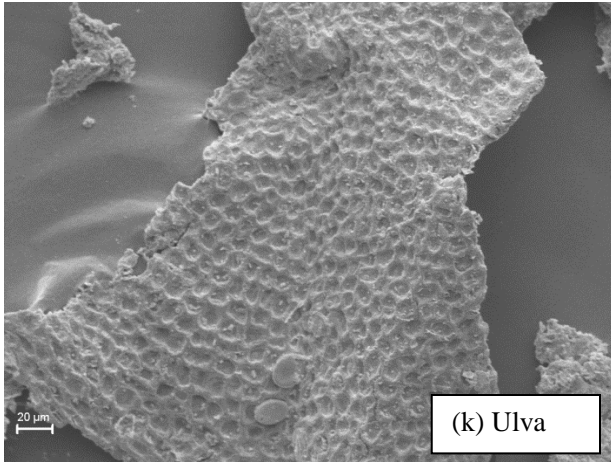
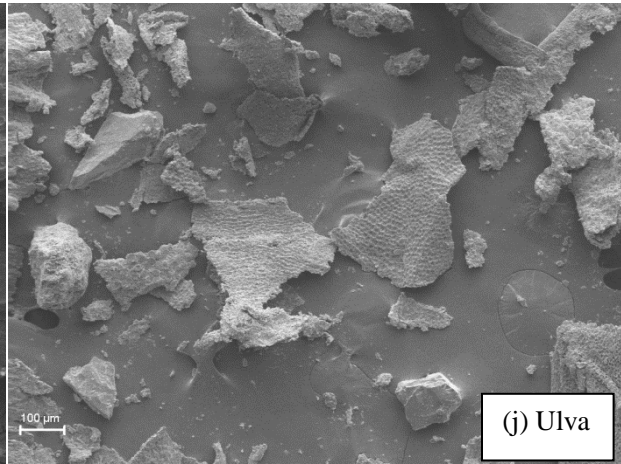
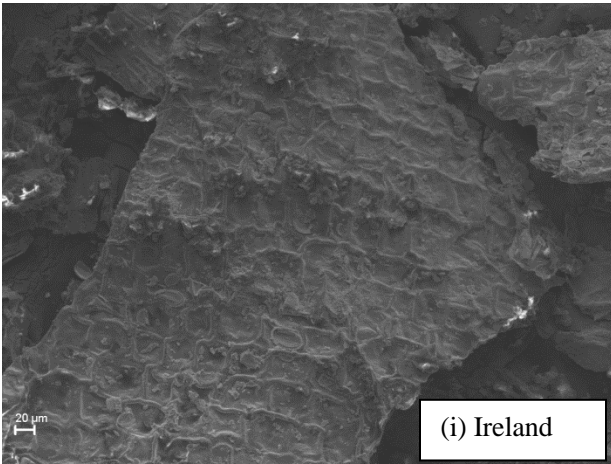
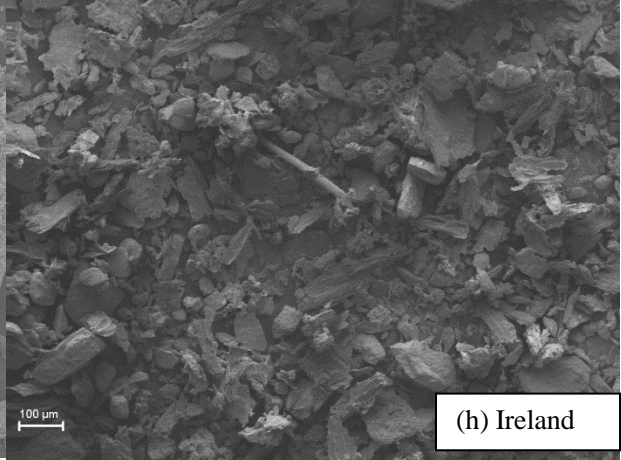
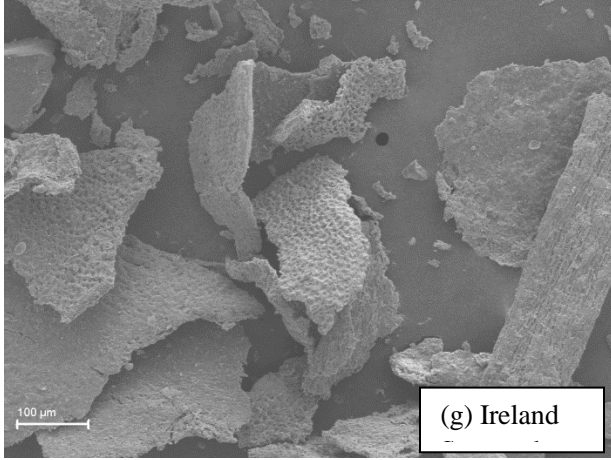
(d) AlgaEvolve

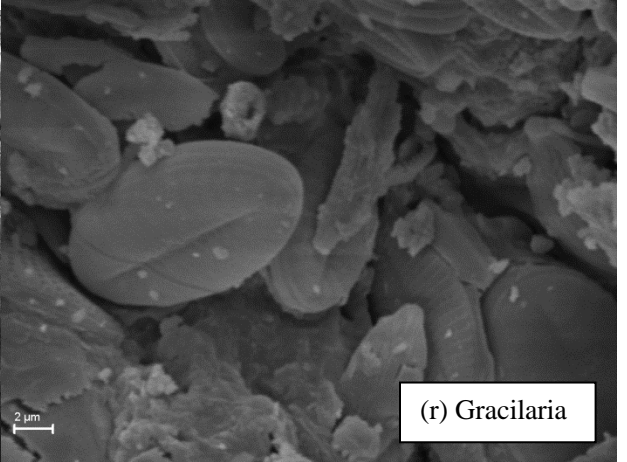
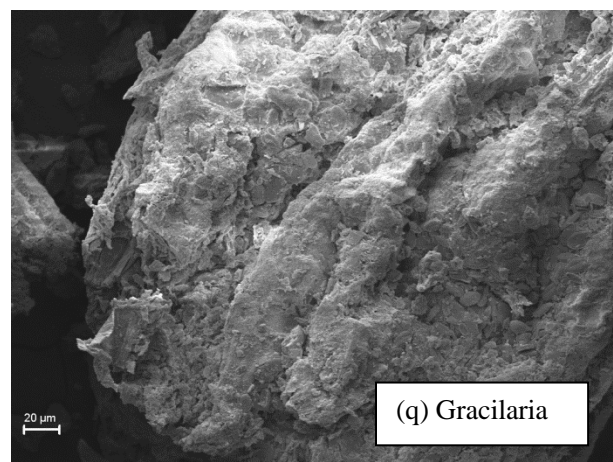
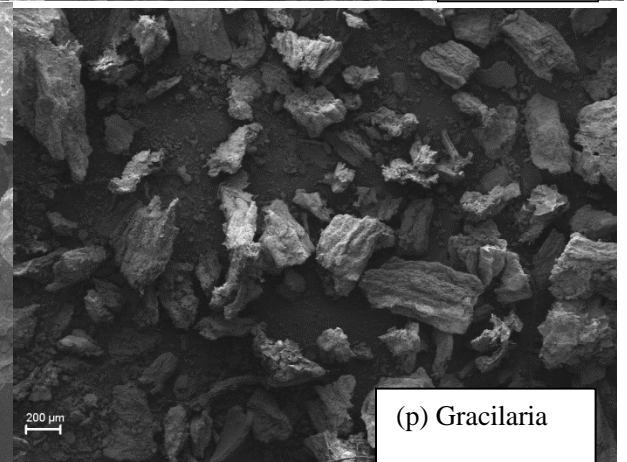
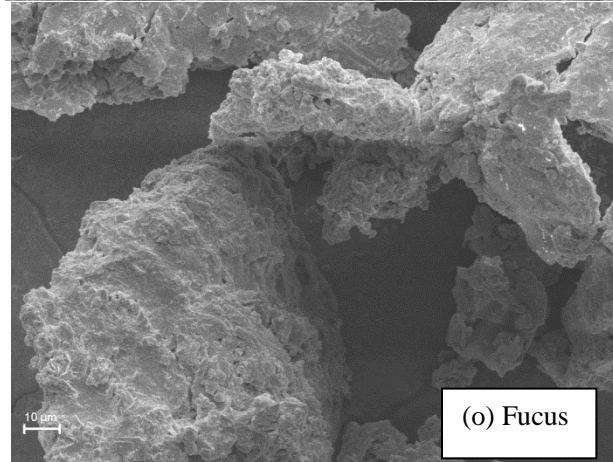
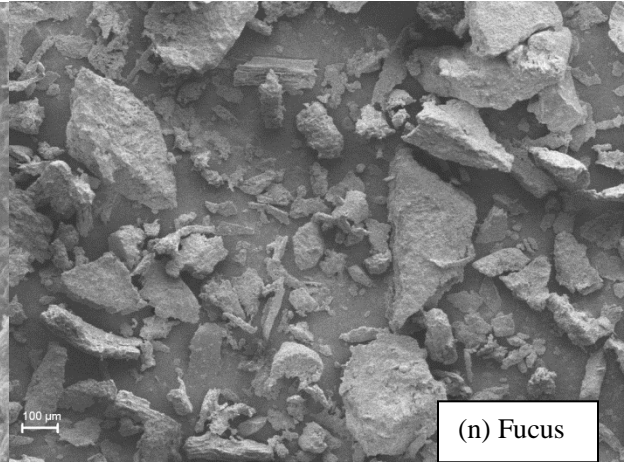
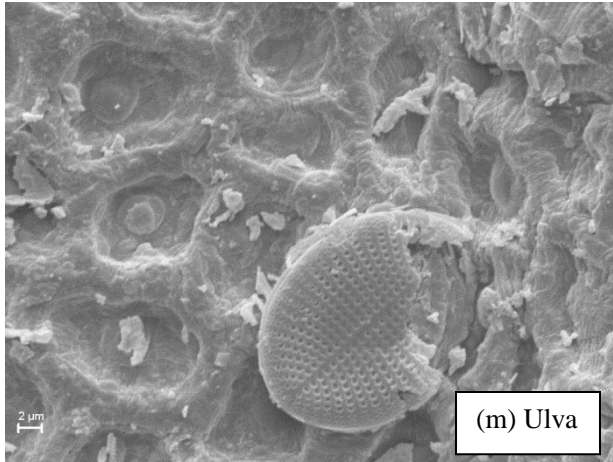


(e) GO2Algae



(f) GO2Algae





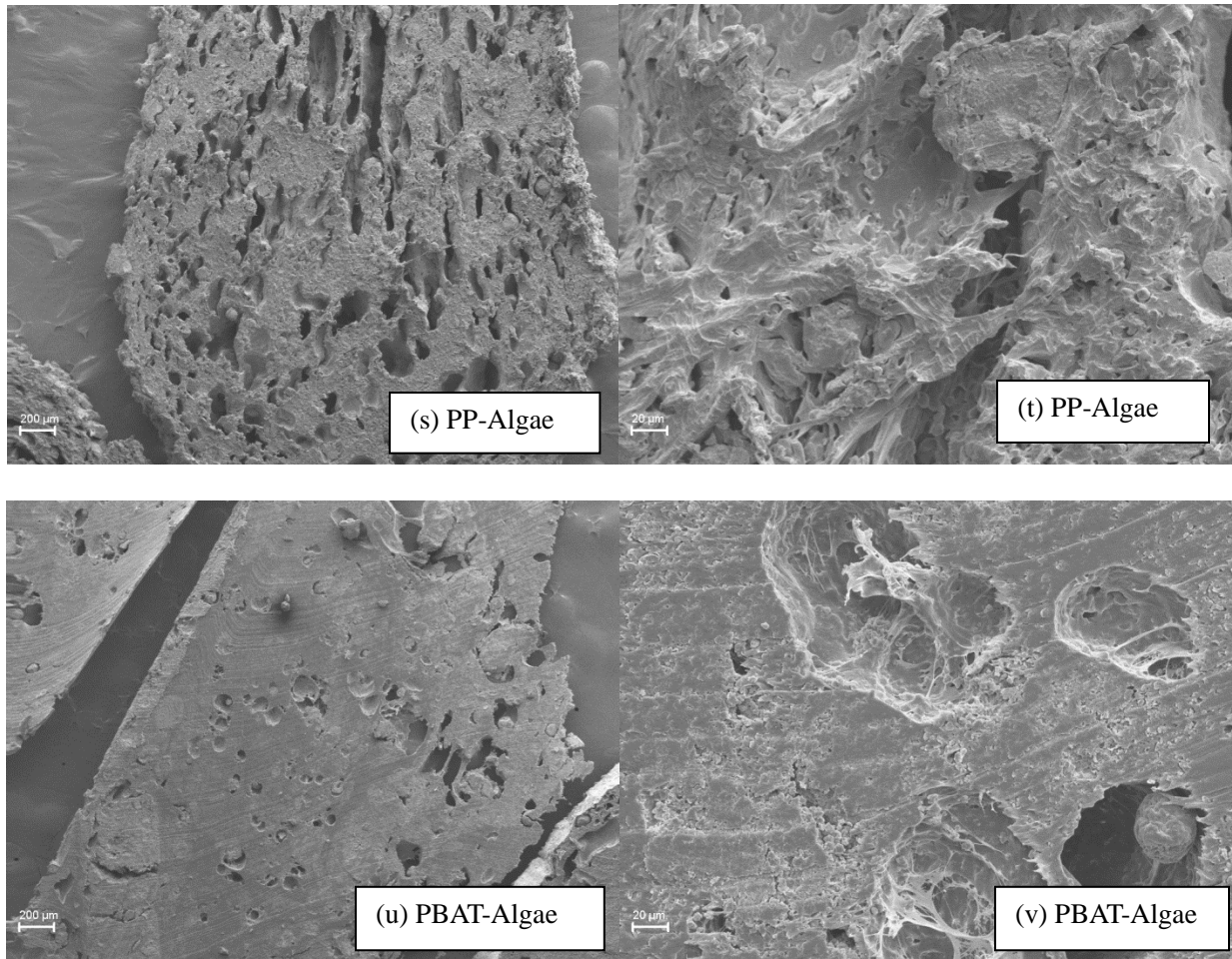


Figure 2.1 Scanning Electron Microscopy of Algae Samples

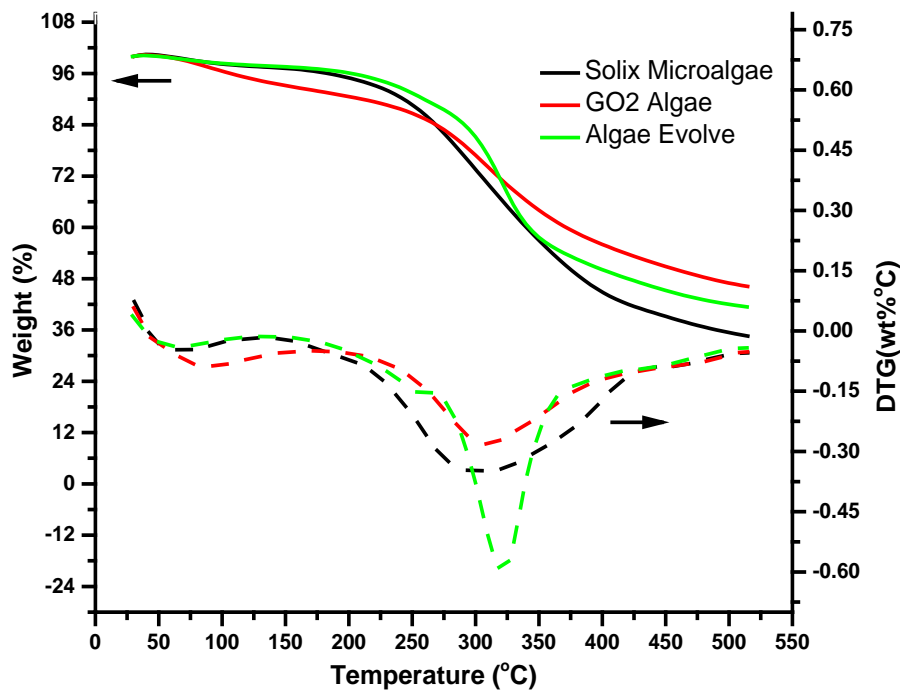
Thermal Analysis of Algae Biomass

For the thermal analysis, TGA and DSC were performed on all the samples with results shown in Figure 2.2 and Figure 2.3, respectively.

The TGA results of the micro algae show two-step degradation (Figure 2.2). The first one is around 50-100°C which represent the bound water and low volatiles loss. The other is around 300°C. The temperature range from 250-350°C represents carbohydrate and protein burning since it occurs in the range where carbohydrates (e.g., hemicelluloses, cellulose, and starch) are typically degraded [27].

The TGA results for macro algae also show a two-step degradation. The weight losses at 50-100°C which demonstrate bound water loss among the samples appear quite consistent. But the temperatures differ for the second step weight losses. Generally, they appear around 230-320°C among which Ireland seaweed varies a lot from the others. The temperature ranges of 250-300°C and 300-350°C are burning of protein and carbohydrate take place, respectively. The deviation of the Ireland seaweed from other species is interesting, notably the difference in degree of the two-step weight loss of Ireland Seaweed over the temperature range of 230-320°C.

The algae-based polymer samples have a three-step degradation. The first is around 50°C representing a bound water loss. The second is about 280°C. The first two steps of degradation are of tiny scale when compared to the third one. The third degradation of the PP algae is about 400°C while that of the PBAT algae is about 470°C which may be due to the degradation of the Poypropylene and poly(butylene adipate-co-terephthalate).



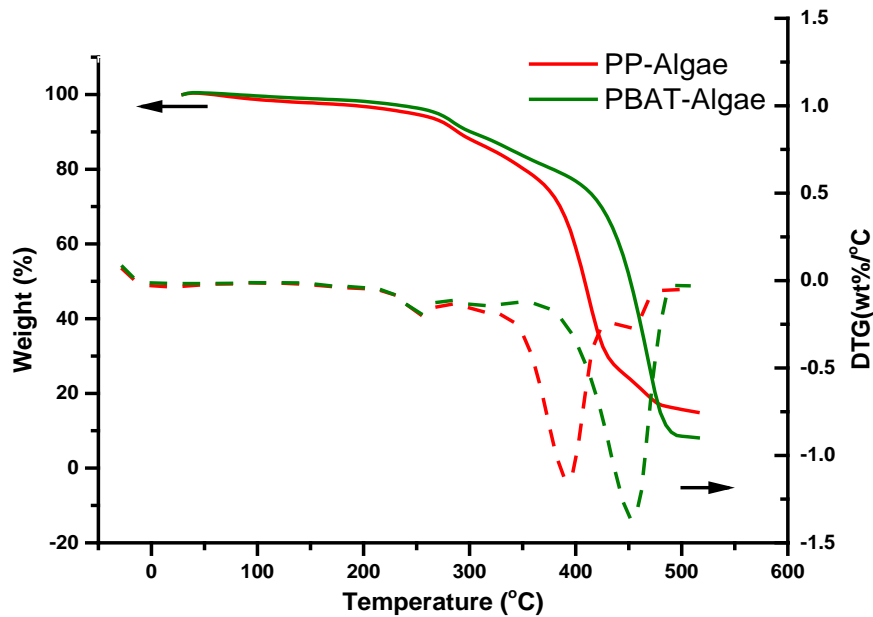
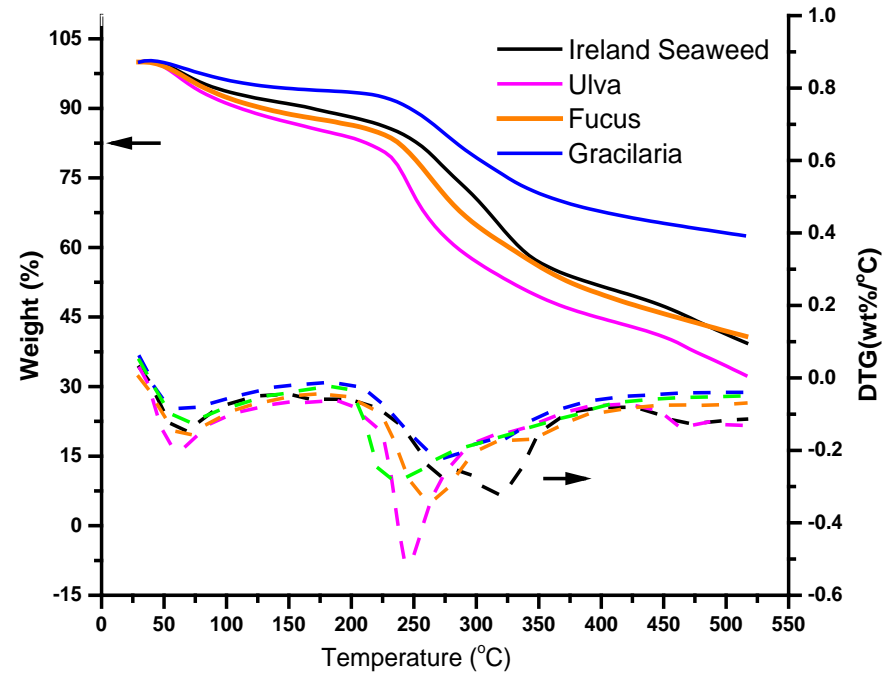


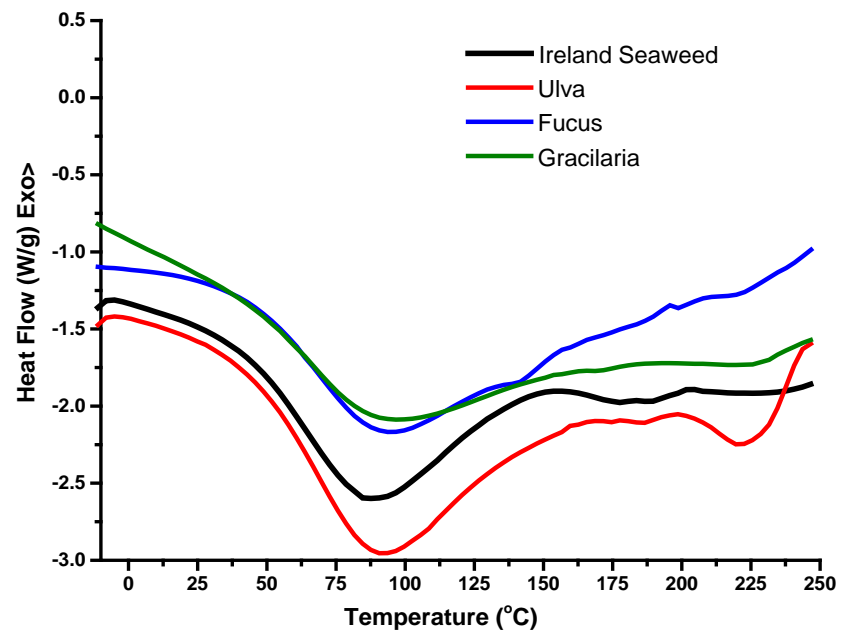
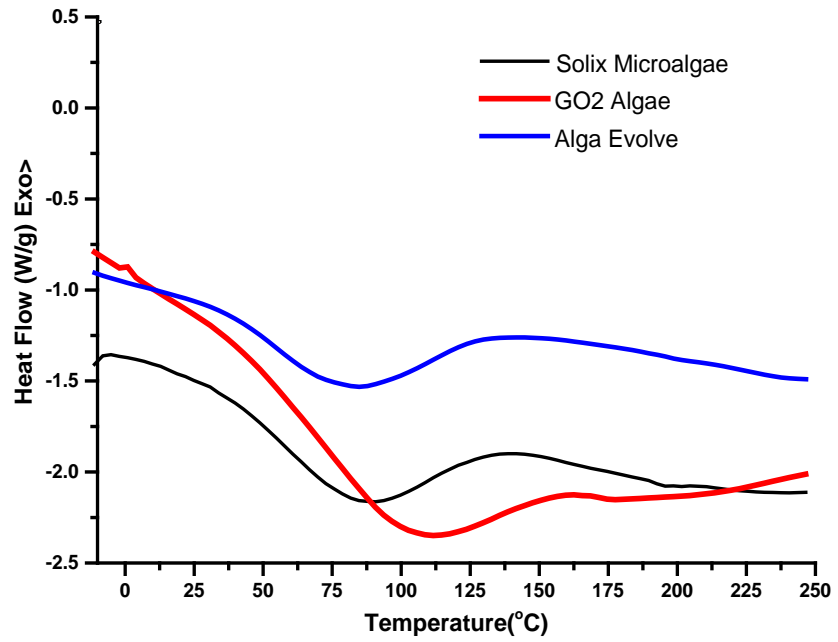
Figure 2.2 TGA of Algae Samples

For the DSC results, all the algae raw material including micro- and macro- algae, most of the strong endothermic peaks of the DSC results are below 100°C (Figure 2.3) although around 100°C, the strongest endothermic peak for the microalgae differs a bit from each other.

AlgaEvolve has a wider range of endothermic process with the peak being about 80°C; the peak of Solix Microalgae appears about 95°C; the peak of GO2 Algae comes only after 100°C. They may be all due to the bound water loss despite the species difference.

The macro algae all show strong endothermic peaks at about 90°C. It is quite interesting that the bound water loss in macro algae seems to be very consistent although the species vary. For Ireland seaweed, the wide slight dip around 180°C may due to the amorphous cellulose hydrolysis which usually appears in this range [28]. Another thing worth noting is that Ulva has a strong endothermic peak at around 225°C. It occurs in the range when carbohydrate and protein usually burn, so it is understandable that Ulva having this strong peak considering Ulva has a much higher carbohydrate content than other species.

The DSC results of the algae-based polymer samples are quite different from those of the raw material. PP-Algae has two endothermic peaks: one is around 100°C and another one shows until 165°C. They may represent some bound water loss within algae and the melting of Polypropylene, respectively. The melting of Polypropylene is usually seen from 147-176°C. PBAT-algae, on the other hand, just have the peak at 165°C. The thermoplastic blends from algae may be more cost effective and degradable than the traditional thermoplastics. Polypropylene (PP) is a thermoplastic polymer used in a wide variety of applications including packaging and labeling, textiles, stationery, plastic parts and reusable containers of various types. Poly(butylene adipate-co-terephthalate) (PBAT) is a biodegradable polymer with high ultimate elongation but low modulus [29].



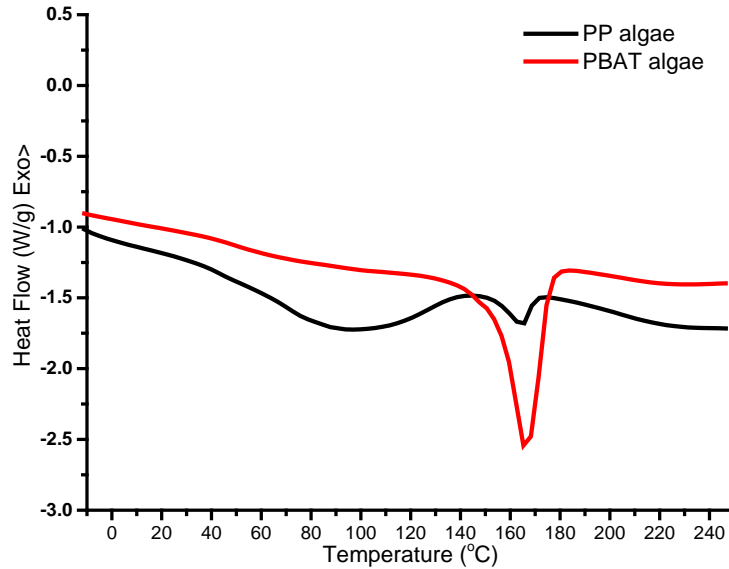


Figure 2.3 DSC of Algae Samples

Dynamic Mechanical Analysis of Algae Biomass Plastics

Ireland seaweed plastic formulation of 100% bio based and hybrids that were blended with water were tested on the DMA system. The DMA measurements help determine the viscoelastic behavior by evaluating the elastic modulus G' (or storage modulus), which is related to the stiffness of the material, and the viscous modulus G'' (or Tan Delta), which is related to the potential of energy absorption in the sample [16]. All DMA results shown represent an averaging of duplicate DMA test runs. Figure 2.4 shows the flexbars of Ireland Seaweed plasticized with water. All of the samples aside from pure Ireland seaweed appear to have similar results, showing a substantial effect from plasticization with water.

Flaxbars of pure Ireland Seaweed and Solix Microalgae, and Ireland Seaweed blended with water were also fabricated. However, except for the pure Solix Microalgae flaxbars, others that were blended with water were damp and easy to stick to the mold after cooking and cooling under pressure and broke easily when taking out of the mold. This may be because compared to Ireland Seaweed, Solix Microalgae contain less fiber which may stiffen the material. Generally,

with addition of plasticizer, flexibility and extensibility of the material was increase while at a molecular level, tensile strength and stiffness would increase. But when the plasticizer was added above the optimum ratio, the softening effect would dominate. The best ratio is affected by the properties of both the material and plasticizer. Thus, in the case of Solix Microalgae, water as plasticizer may be softening the blend even at 10%. The results of the thermal analysis indicate that algae have the potential of developing bio-plastic whose properties depend on the nature of the algae material and the best ratio of biomass to plasticizer may vary.

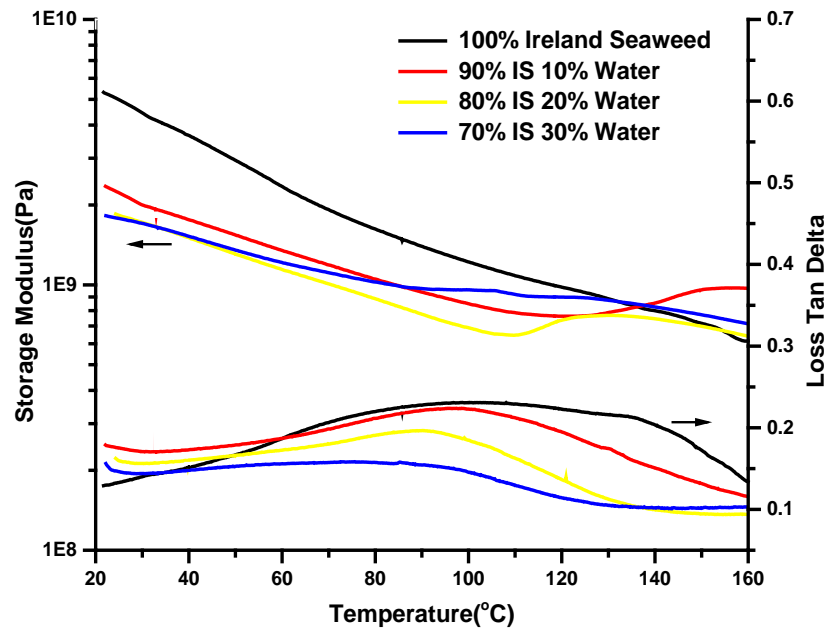


Figure 2.4 DMA of Flexbars of Ireland Seaweed Plasticized with Water

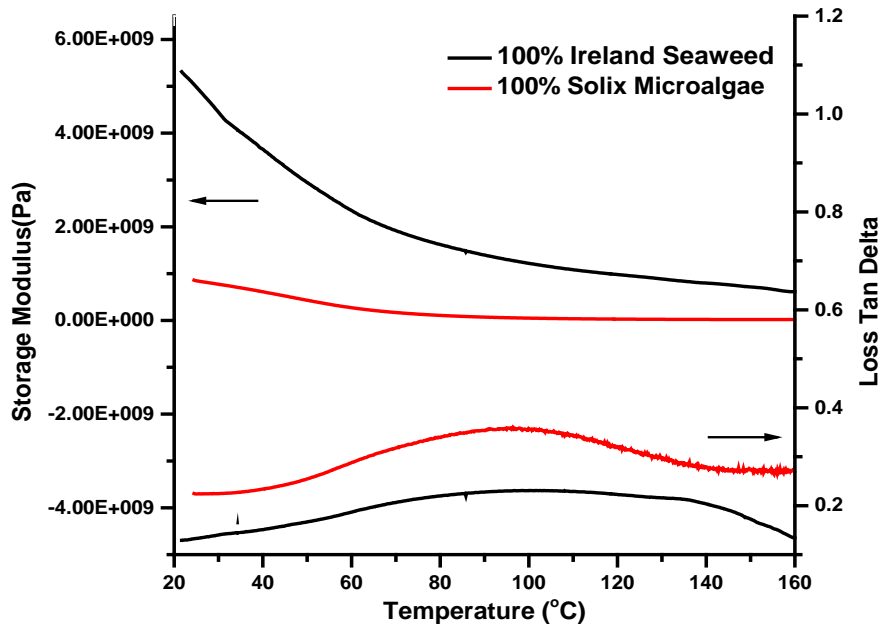


Figure 2.5 DMA of Flexbars of Solix Microalgae and Ireland Seaweed

DMA results of testing of flexbars of 100% Ireland Seaweed and those of 100% Solix Micro are compared in Figure 2.5. Perhaps water is not good for the plasticization of Solix Microalgae. From the graph above, it can be seen that flexbars made of Ireland Seaweed have a higher modulus which means higher stiffness, while those made of Solix Microalgae show higher Tan Delta which means better energy absorption.

Conclusion

Proximate composition analysis of the raw material showed that the microalgae contain more fixed carbon and protein than the macroalgae. DMA of flexbars of Ireland Seaweed blended with water showed a substantial effect from plasticization with water. Flexbars made of Ireland Seaweed have a higher modulus which means more stiffness, while those made of Solix Microalgae show higher Tan Delta which means better energy absorption.

CHAPTER 3

PLASTICIZATION OF SPIRULINA MICROALGAE BIOMASS

Experiments

A certain weight of Spirulina (SP) algae biomass powder was plasticized by Ethylene Glycol (EG) at weight ratios from 10% to 30% at 5% intervals. These mixtures were used for making flexbar and their thermal and mechanical properties were characterized as described in Chapter 1.

Results and Discussion

Thermal Analysis of Spirulina Plasticization Bio-plastics

Figure 3.1 shows the TGA of dry Spirulina powder, the Spirulina showed a two-step degradation, the first one starting at around 25 °C and ending at about 130 °C, which represents bound water and low volatile loss. The second one starting at around 200 °C and ending at about 380 °C, might be from the carbohydrate and protein degradation.

The DSC of dry Spirulina powder shows the denaturation peak begins at around 40 °C and ends at about 150 °C as can be shown in Figure 3.2. The main peak for dry Spirulina powder is around 100 °C. At 150 °C, Spirulina proteins are maximally denatured.

The TGA result indicates that degradation can occur from around 175 °C, while the DSC result indicates that proteins are maximally denatured at 150 °C. Therefore, the Spirulina should be processed at 150 °C which does not risk its degradation at higher temperatures, yet meanwhile could yield its maximum denaturation.

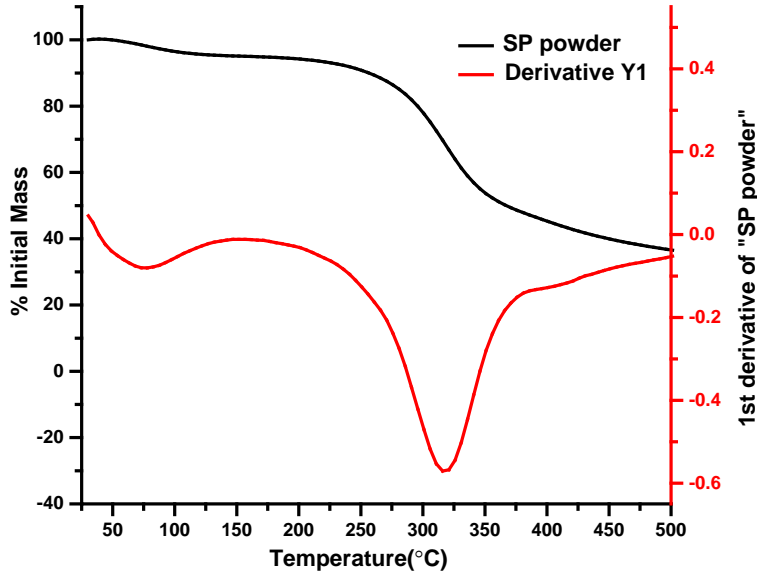


Figure 3.1 TGA of Spirulina Powder

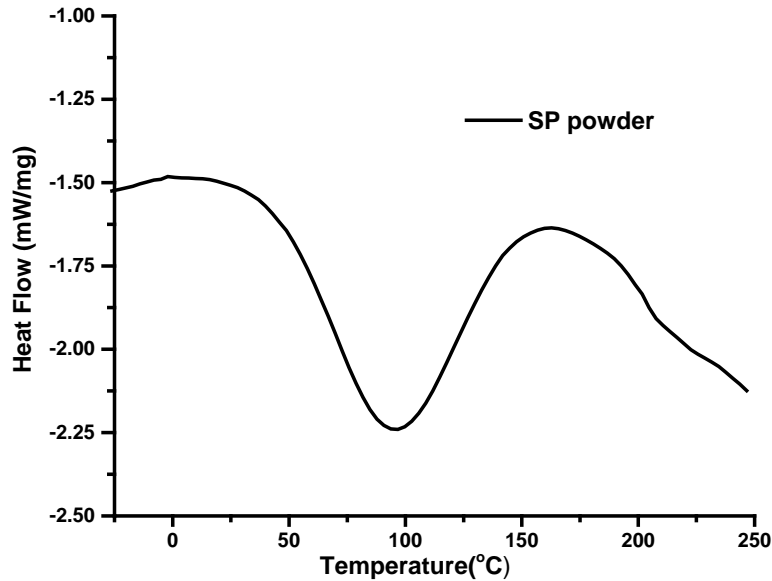


Figure 3.2 DSC of Spirulina Powder

Figure 3.3 shows the TGA of Spirulina plasticized with Ethylene Glycol at different weight ratios varying from 0% to 30% to determine most suitable plasticization ratio of protein and plasticizer. The first weight loss of 25-100 °C are due to bound water loss. The gradual weight loss of the Spirulina- Ethylene Glycol bio-plastics was started from 125 °C and possible halted around 250 °C because of the evaporation of Ethylene Glycol. The Spirulina bio-plastics

show one degradation peak at about 310 °C for all formulations, and this degradation was left shifted in bio-plastics with more Ethylene Glycol making its starting variable from 200 °C to 225 °C, suggesting that the additional Ethylene Glycol aided the degradation and allowed its degradation to start at lower temperatures, which could possibly be the effect of Ethylene Glycol interfering with the interaction between carbon hydrate and protein molecules in the plastic matrix, in turn making these molecules more easily degradable.

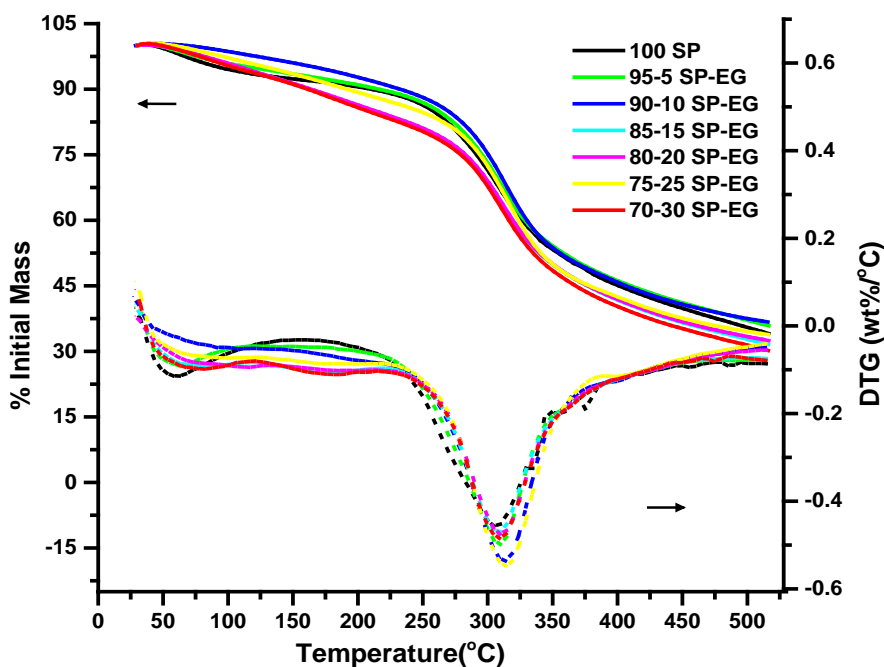


Figure 3.3 TGA of Spirulina Plasticized with Ethylene Glycol

Figure 3.4 shows the DSC of Spirulina plasticized with Ethylene Glycol at different weight ratios varying from 0-30%. For 100% Spirulina bio-plastic and those plasticized by EG weight percentage of 5% and 10%, only one peak representing the SP denaturation at around 75 °C was observed. For the 5%, 10%, 15% EG formulation, this temperature is a little lower than that of the dry Spirulina powder as indicated in Figure 3.2, meaning a plasticization effect of EG which would lost with EG% more than 20%. For the bio-plastics that contain EG from 15-30%, in addition to the 80 °C endothermic peak, another peak at about 165 °C also presents and

increases intensity with higher EG%. This 165 °C peak is due to the evaporation of EG which can happen from 125 °C to 200 °C since EG has flash point of 111 °C and boiling point at 197 °C. As more EG was added, more free plasticizer would present and ready to vaporize. This indicates that EG would evaporate from the bio-plastics from 125-200 °C if EG percent is higher than 10%.

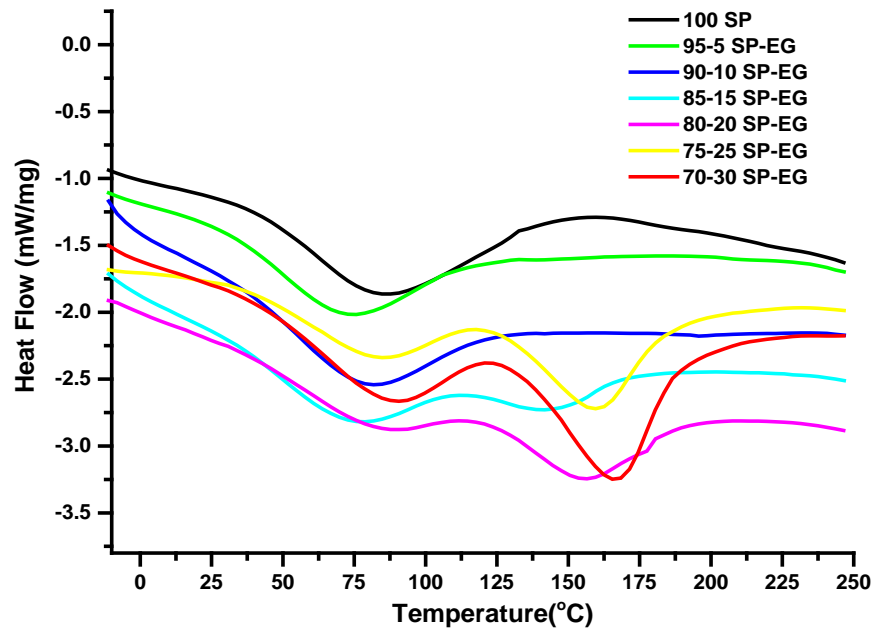


Figure 3.4 DSC of Spirulina Plasticized with Ethylene Glycol

Mechanical Properties of Ethylene Glycol Plasticized Spirulina Bio-plastics

Figure 3.5 shows DMA results of different Spirulina-Ethylene Glycol bio-plastics. The modulus was lowered while the Tan Delta values showed a left shifted pattern with the increasing content of plasticizer. This means with the increase of EG, the bio-plastic become less stiff and has Tan Delta in lower temperature peaks, meaning lower T_g. However, for the 75-25 and 70-30 SP-EG, the transition from glassy to rubbery regions is too broad, and happen at room temperature. Usually, the modulus would decrease exponentially after T_g. Therefore, these two formulations have too low even modulus at room temperature, making them unacceptable for

end use. The 95-5, 90-10, and 85-15 SP-EG formulation have good plasticizing effect, relatively moderate modulus and tan delta. Among these 90-10 has the best balance of modulus and flexibility. Therefore, the 90-10 SP-EG formulation was considered the best.

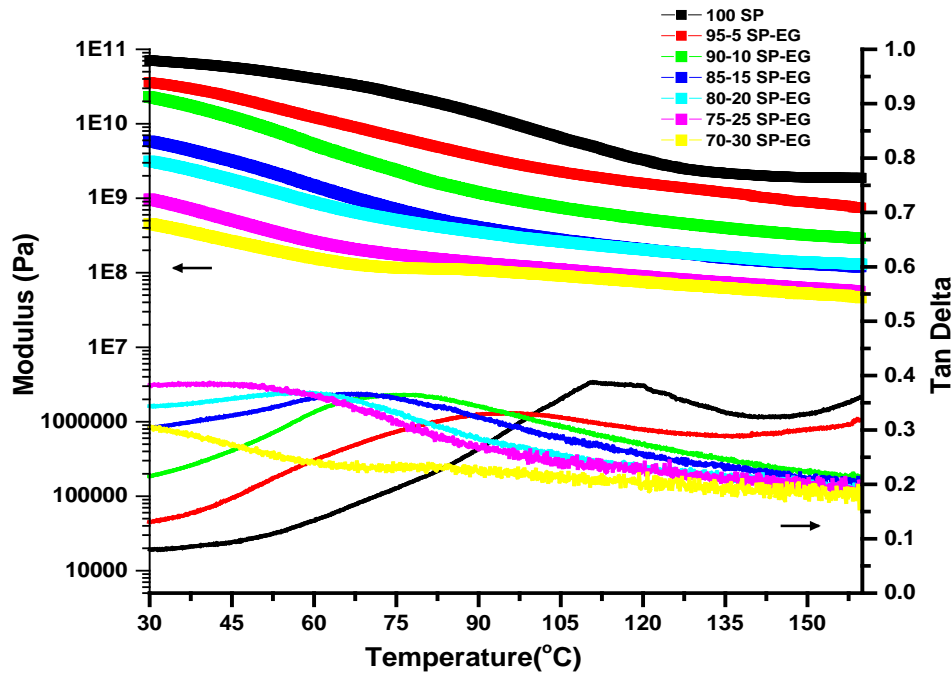


Figure 3.5 DMA of Flexbars of Spirulina Plasticized with Ethylene Glycol

Figure 3.6 shows the tensile properties of Spirulina bio-plastics plasticized with Ethylene Glycol. Because the power of the pneumatic jaws could not be adjusted, some samples (i.e., with no PE blended in the formulation) are broken at the jaws, the PE sample slipped out from the jaws before it broke in the middle. However, the tensile testing results give a general idea of how the samples behave under stress. The most obvious conclusion from these data is that 95-5 SP-EG is better as a bio-plastics which has a good balance of load and extension characteristics, compared to the 100%SP which has high extension at maximum load but low maximum load at break, and the 90-10 SP-EG which has high maximum load at break but low extension at maximum load. This can be further confirmed by the load-extension curve.

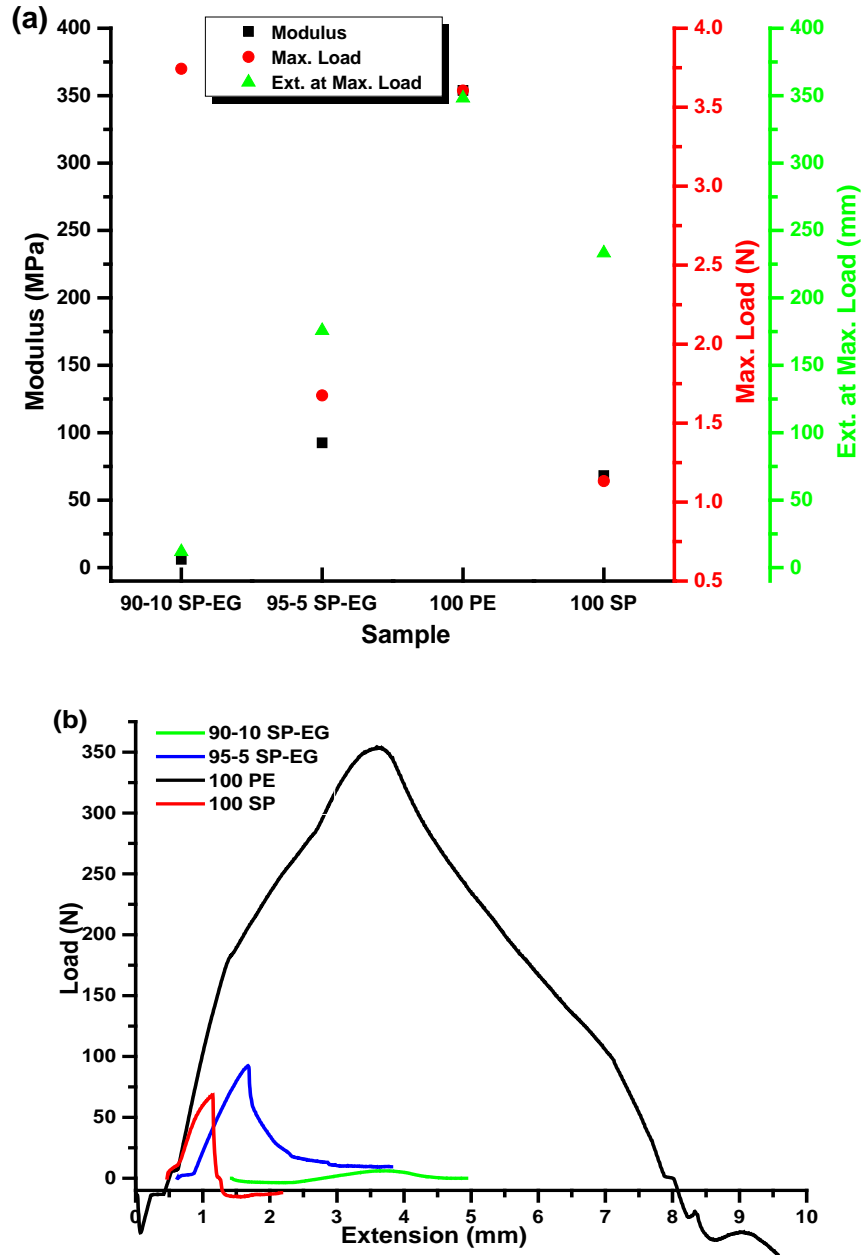
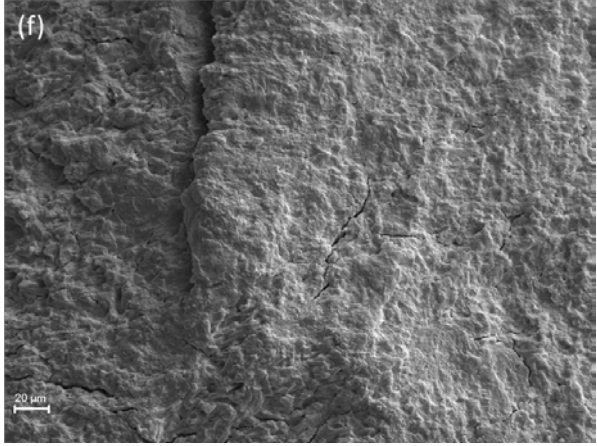
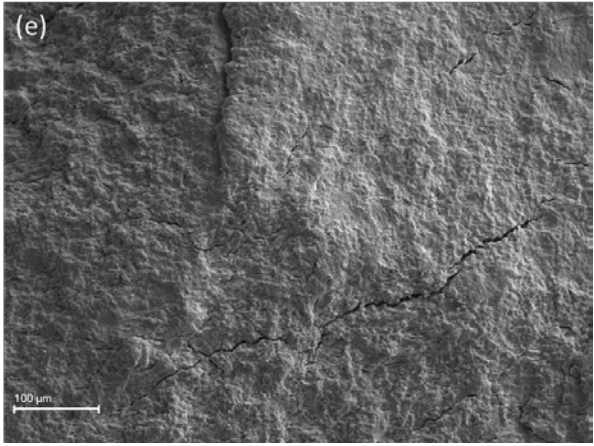
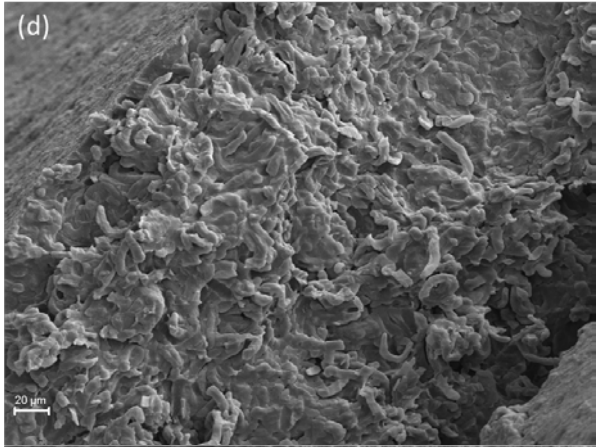
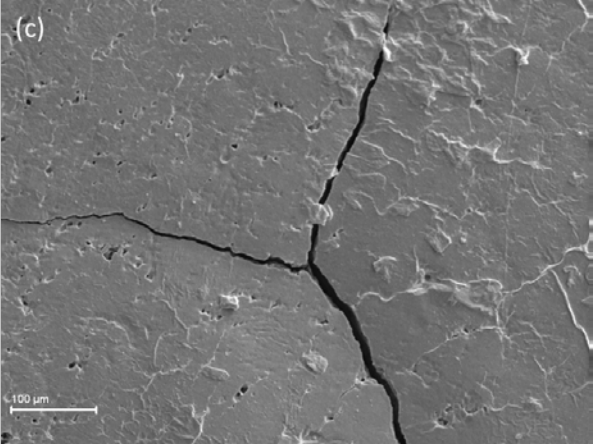
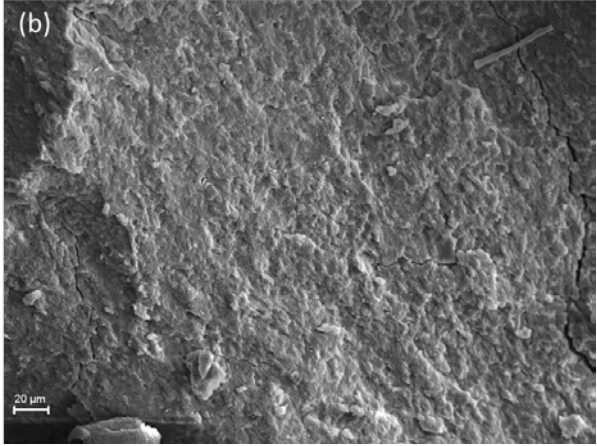
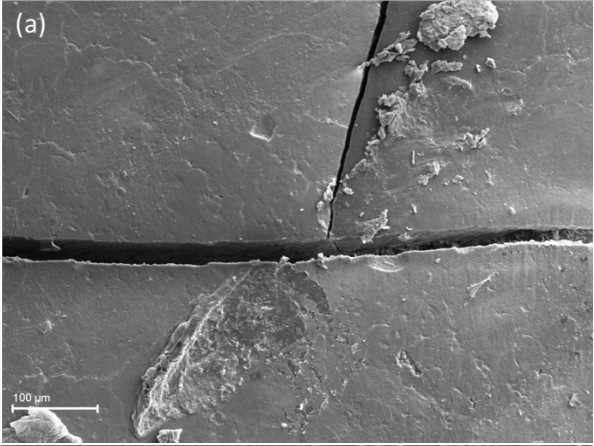


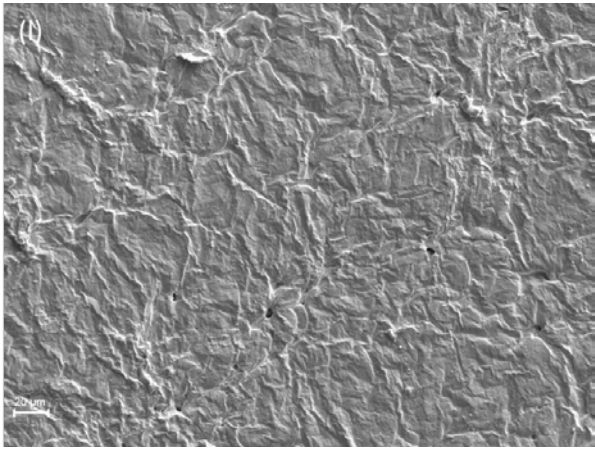
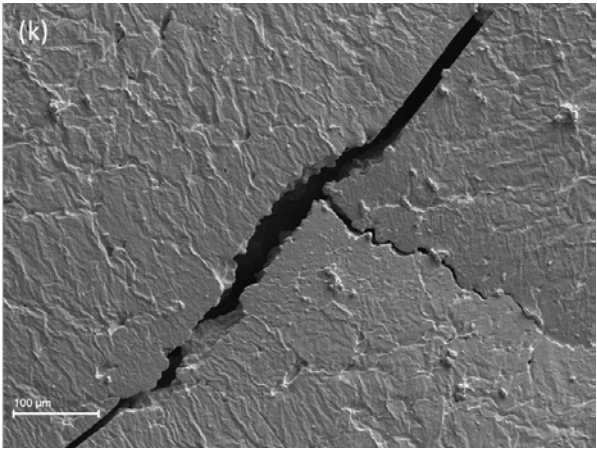
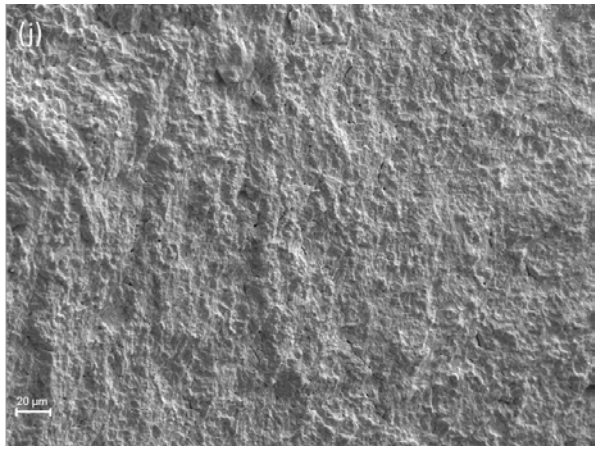
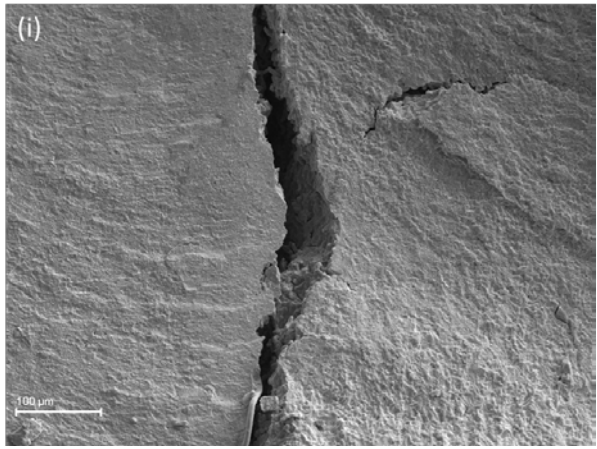
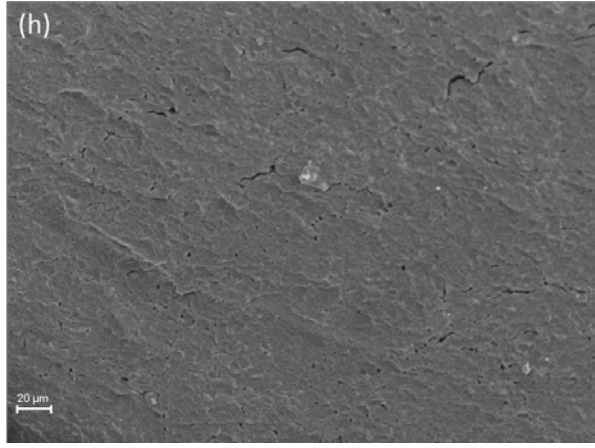
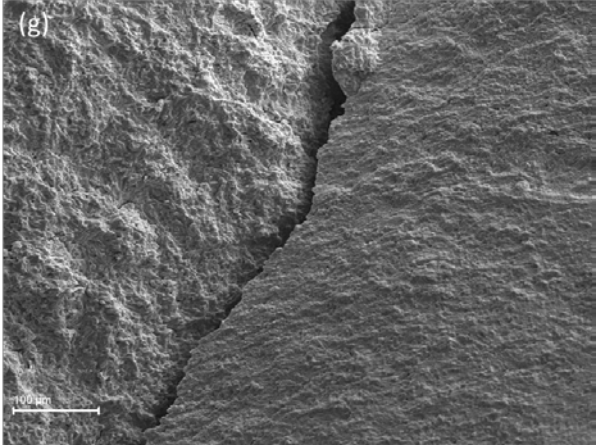
Figure 3.6 Tensile Properties of Spirulina Plasticized Bio-plastics

Morphology of Spirulina Thermoplastic Blends

Figure 3.7 shows the morphologies of fracture surfaces of different formulas of Ethylene Glycol plasticized Spirulina bio-plastics. The magnification of the left sided pictures are set as $\times 500$ to get an overview of the surface, while that of the right sided pictures are set as $\times 1000$ to

get finer images of the ridges or the surface. The 100% SP and 95-5 SP-EG formula have generally smooth surfaces but rough ridges. This may indicate more toughness in the material because toughness increases the rough nature of the break [16]. The 90-10 SP-EG formula shows no obvious cracks on the surface and the 85-15 SP-EG formula has smooth surfaces indicating a good phase dispersion between Spirulina and the plasticizer and higher inter-surface adhesion. But still, 90-10 SP-EG was considered the best due to its good morphological consistency. For the 75-25 SP-EG and 70-30 SP-EG, there are many large cracks or holes on the surface of the plastics, which was result in both lower modulus and Tan Delta.





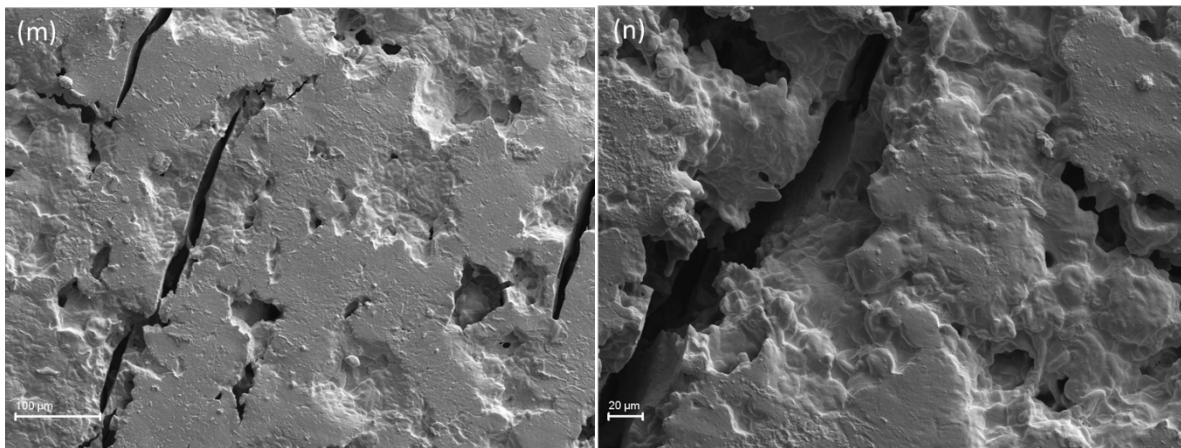


Figure 3.7 SEM of Ethylene Glycol Plasticized Spirulina Bio-plastics

(a), (b) 100 SP; (c), (d) 95-5 SP-EG; (e), (f) 90-10 SP-EG; (g), (h) 85-15 SP-EG; (i), (j) 80-20 SP-EG; (k), (l) 75-25 SP-EG; (m), (n) 70-30 SP-EG;

Conclusion

Different weight ratio of Ethylene Glycol plasticized Spirulina bio-plastics were made evaluated by thermal, mechanical and morphological properties. The TGA results of the Spirulina bio-plastics shows the degradation peak at about 310 °C consistent well with that of its dry powder, and the adding plasticizer Ethylene Glycol aided the degradation and allowed its degradation starting at a lower temperature, which resulted from the interaction between carbohydrates and protein molecules was interfered by Ethylene Glycol.

The DSC data show two endothermic peaks. For the 5%, 10%, 15% EG formulation, the SP denaturation peak at around 75 °C is left shifted than that of the dry Spirulina powder as indicated in Figure 3.2, meaning a plasticization effect of EG which would lost with EG% more than 20%. The 125-200 °C peak indicates that EG would evaporate from the bio-plastics from 125-200 °C if the EG% is higher than 10%.

DAM shows that with the increase of EG, the bio-plastic become less stiff and has lower Tg. However, for the 75-25 and 70-30 SP-EG, the transition from glassy to rubbery region are too broad and happen at room temperature making them unacceptable for end use. The 95-5, 90-10, and 85-15 SP-EG formulation have good plasticizing effect, relatively moderate modulus and tan delta and among which 90-10 has considered the best balance of modulus and flexibility.

The Instron tensile testing showed that 95-5 SP-EG bio-plastic has better load and extension but the Instron this may need further experiments to justify due to the inconsistency of the results.

The fracture surfaces images from SEM indicate that bio-plastic of 90-10 SP-EG formula shows no obvious cracks on the surface and its good morphological consistency indicating it has a well phase dispersion between Spirulina and the plasticizer and higher inter-surface adhesion.

CHAPTER 4

BLENDING OF SPIRULINA MICROALGAE BIOMASS

Experiments

The Ethylene Glycol (EG) plasticized Spirulina (SP) biomass was blended with ultra-high molecular weight polyethylene (UHMW-PE) with different PE weight ratios of from 20% to 80% at 15% intervals. These mixtures were used for making flexbar and their thermal and mechanical properties were characterized as described in Chapter 1.

Because the performance of 90-10 SP-EG formulations were regarded as the best, a ratio of 1:9 Ethylene Glycol to Spirulina was used to determine the EG quantity needed for hybrid algae polyolefin blends. The quantity of EG needed to plasticize a quantity of algae in blends was determined by eq. (1). The carrying capacity of polyethylene for EG was considered the same as PE to glycerol which is 13.33 [16], and the ratio of ethylene glycerol to microalga of 1/9, as shown above, represents the preferred plasticization in a 90:10 ratio. Thermoplastic blends with 20%, 35%, 50%, 65%, and 80% polyethylene were made for DMA analysis, and the amount of glycerol and microalgae needed for formulations was determined by using eq. (1).

Quantity of Ethylene Glycol = (Grams of polyethylene)(1/13.3)+Grams of Spirulina)(1/9)

(1)

Results and Discussion

Thermal Analysis of UHMW-PE Blended Spirulina Bio-plastics

Figure 4.1 shows the TGA of Spirulina and Ethylene Glycol thermoplastic blends after blending with ultra-high molecular weight polyethylene (UHMW-PE). The Spirulina thermoplastic blends showed the polyethylene degradation peak around 480 °C, and Spirulina degradation is shown in thermoplastic blends occurring between 225 °C and 375 °C with a maximum thermal degradation at around 300 °C. Therefore, with the PE% increasing, the Spirulina thermoplastic blends end up with less weight loss at 300 °C and more at 480 °C.

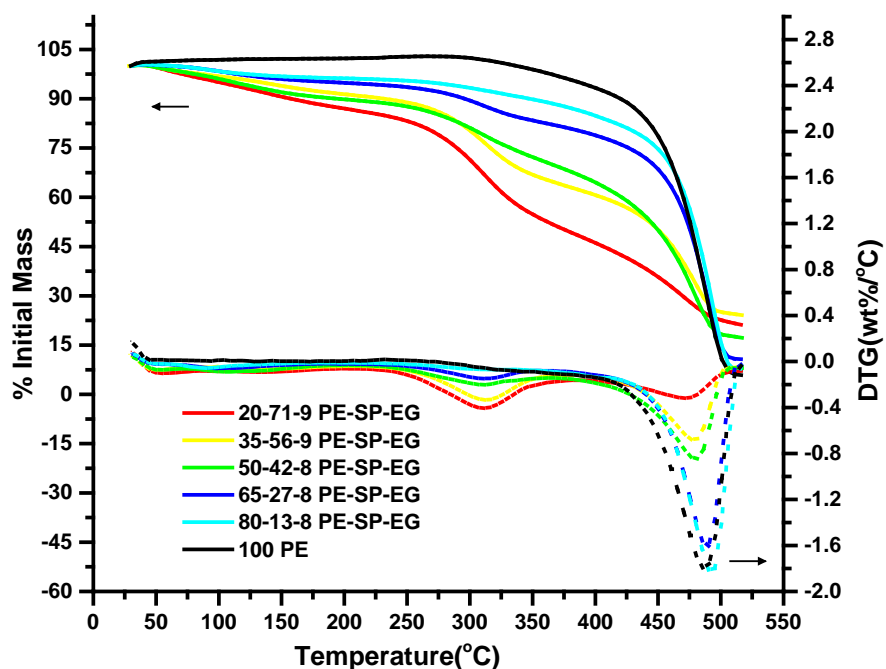


Figure 4.1 TGA of Flexbars of Spirulina Blended with UHMW-PE

Figure 4.2 shows the DSC data of Spirulina and Ethylene Glycol thermoplastic blends after blending with UHMW-PE. The melting point of polyethylene can be observed at around 130 °C generally with increasing peak size as the level of polyethylene increases. The peak at

about 70 °C is because of SP denaturation and its size generally increases as more SP content is present in the formulation. This peak of denaturation can also be confirmed in Figure 3.4.

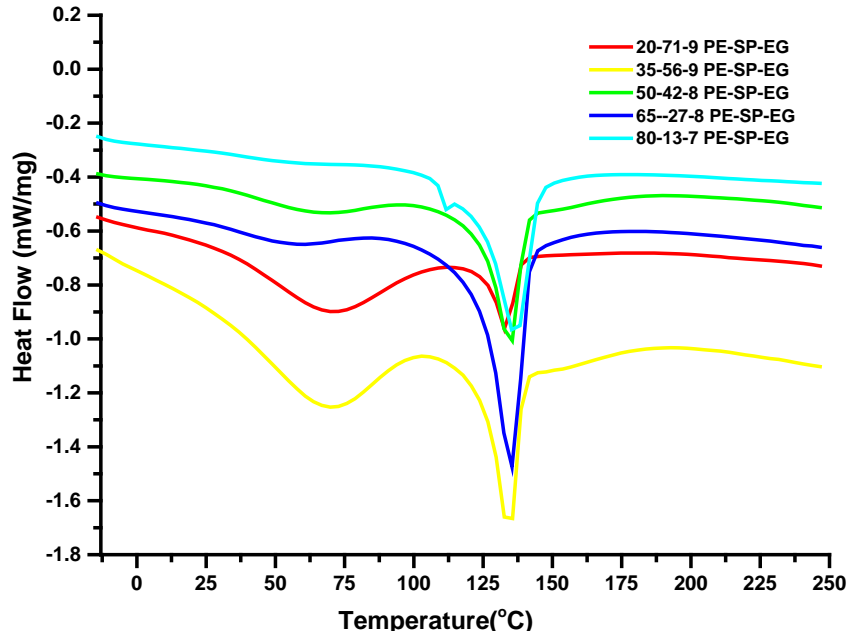


Figure 4.2 DSC of Spirulina Blended with UHMW-PE

Mechanical Properties of Spirulina Thermoplastics Blends

Figure 4.3 represents the DMA of Spirulina and Ethylene Glycol thermoplastic blends after blending with UHMW-PE. There is a shift of peak Tan Delta, from lower temperature to a higher one, and meanwhile an increasing of modulus with the percentage of polyethylene. Formulations that contain more than 50% SP have higher Tan Delta values before 100 °C. But above 100 °C, Tan Delta is higher for formulations that contain more than or equal to 50% PE. The 80% PE formulation have highest modulus values other than 100% PE and even higher Tan Delta values than PE, suggesting this is the best PE blended Spirulina bio-plastic formulation.

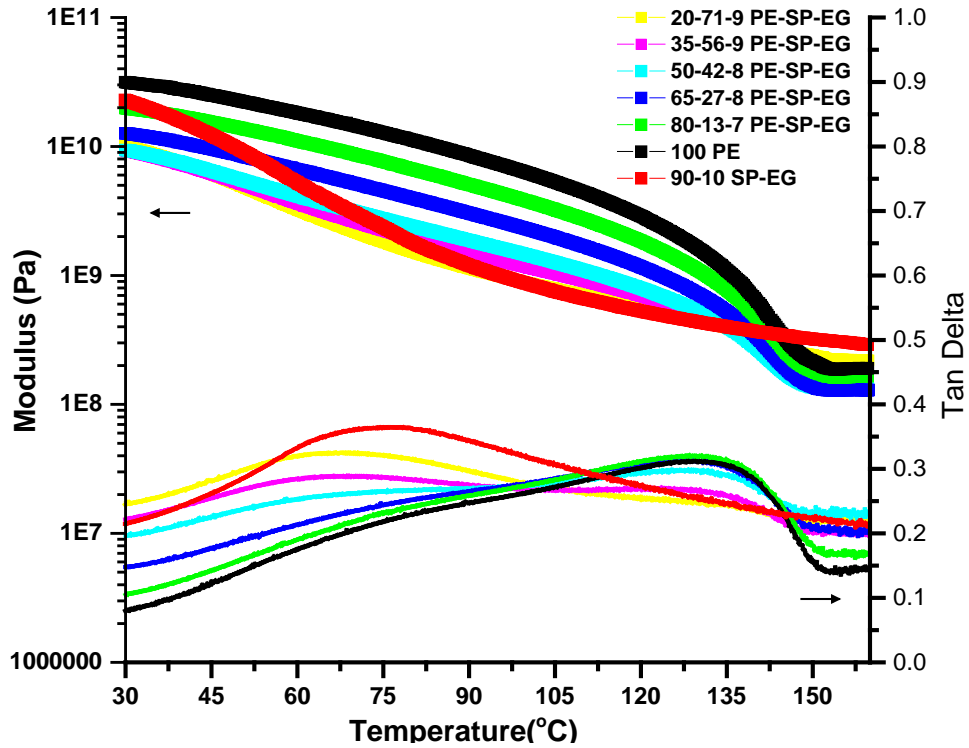


Figure 4.3 DMA of Spirulina Blended with UHMW-PE

Figure 4.4 shows the tensile properties of Spirulina thermoplastics blending with UHMW-PE. The most obvious conclusion from these data is that 80-13-7 PE-SP-EG is a better bio-plastic blend because it has better load and extension characteristics than all the other blended bio-plastics, but its tensile curve actually not comparable to that of 100% PE since PE samples were slipped out from the jaws before breaking. However, it is still clear to see that 80-13-7 PE-SP-EG has the best tensile properties among all the PE-SP-EG blended bio-plastics. The 80-13-7 PE-SP-EG also demonstrate best load-extension curve.

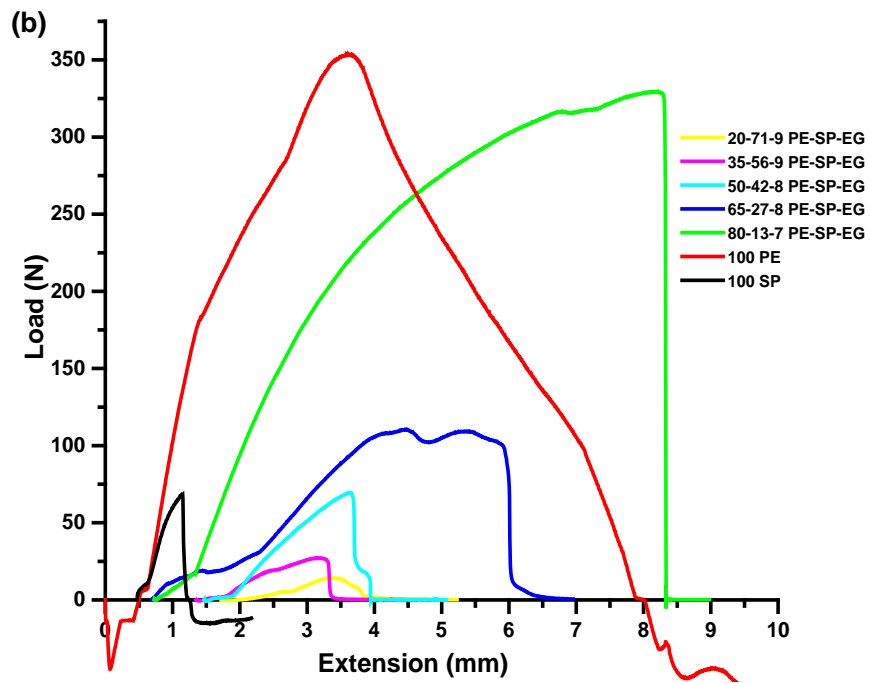
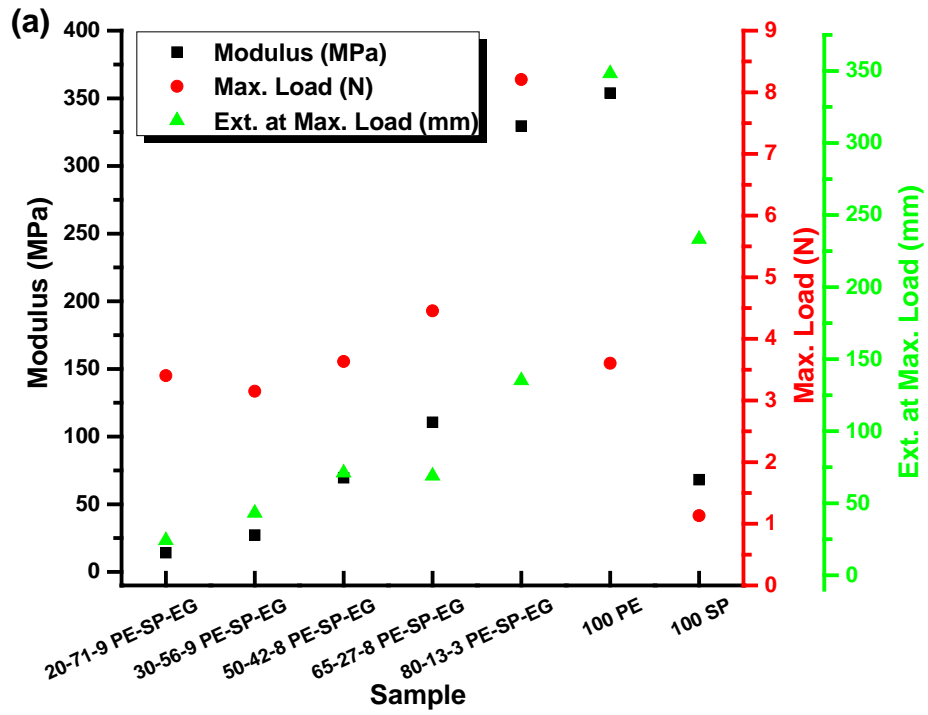
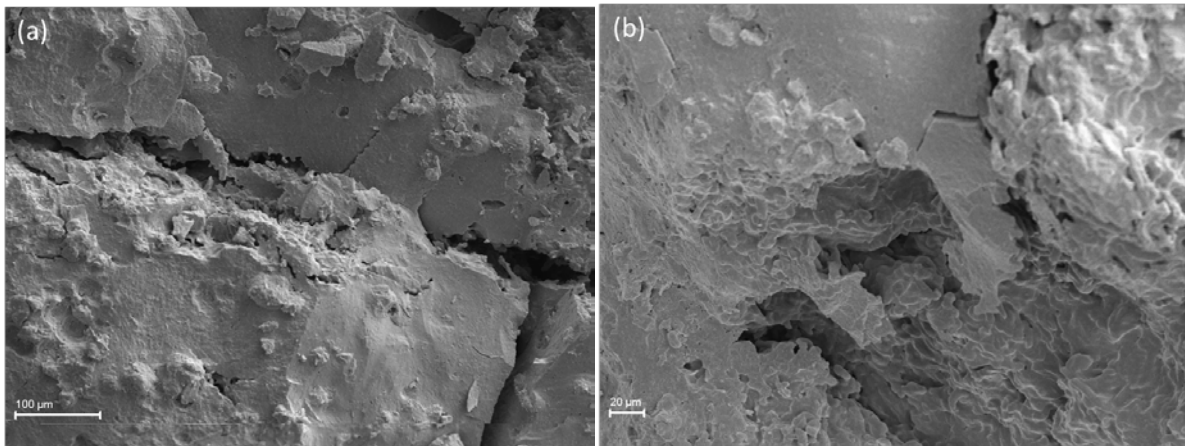
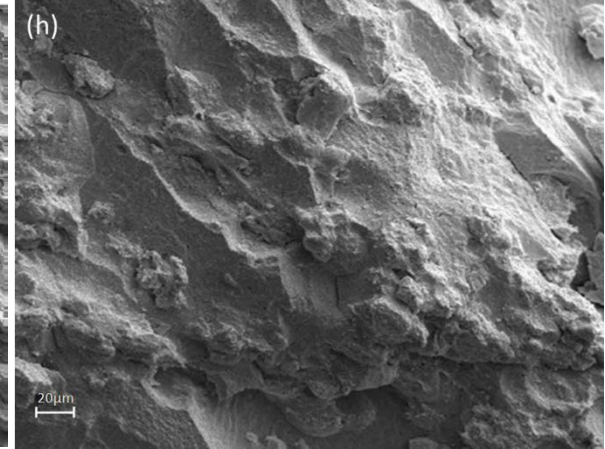
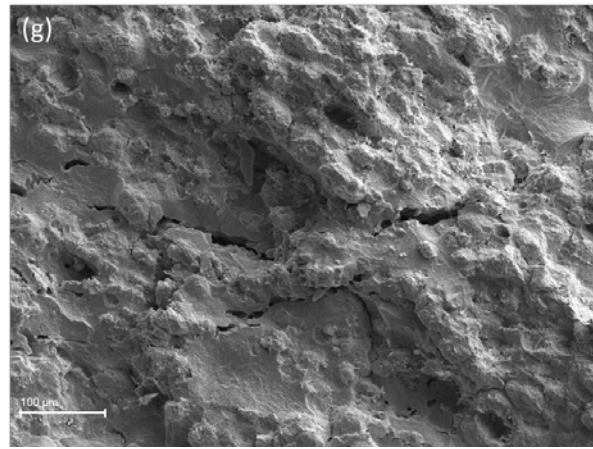
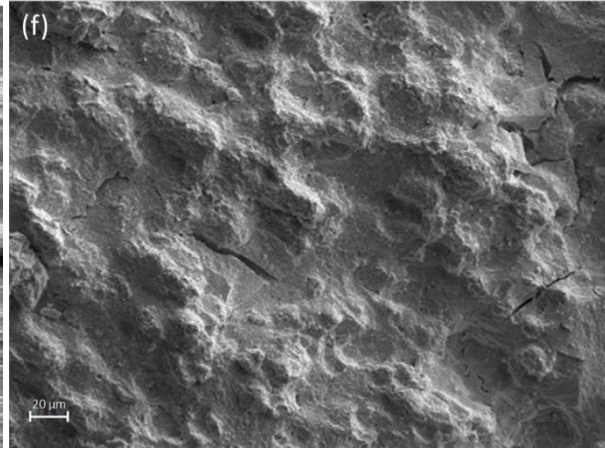
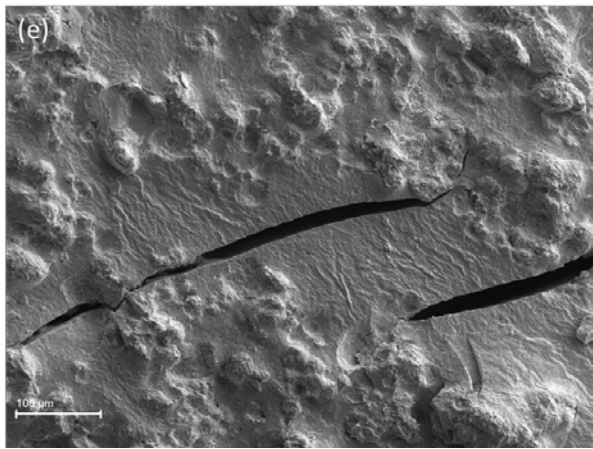
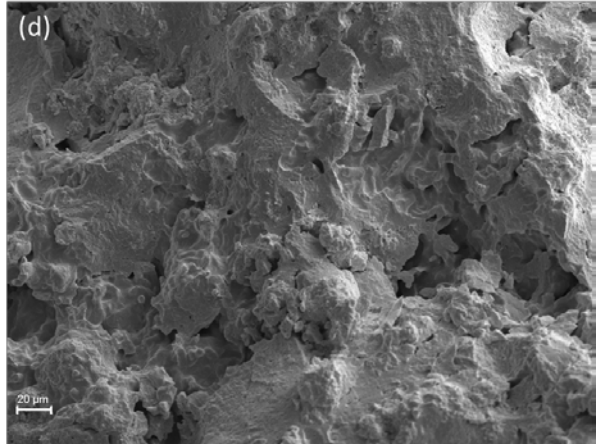
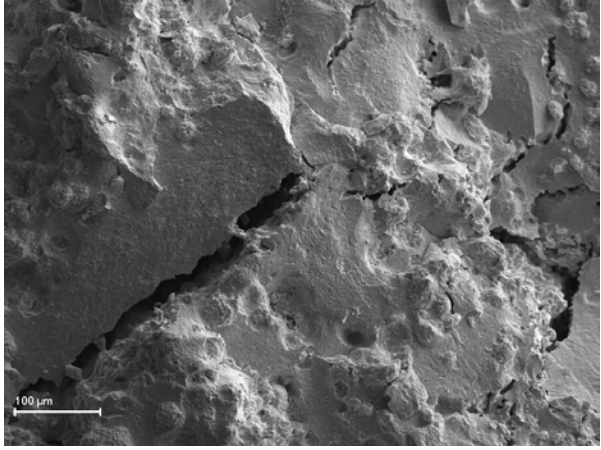


Figure 4.4 Mechanical Properties of Spirulina Thermoplastic Blends

(1) Morphology of Spirulina Thermoplastic Blends

Figure 4.5 shows SEM fracture micrographs of Spirulina thermoplastic blends. Polyethylene is generally seen as the rough areas, while the smooth areas represent Spirulina phase. The surface of thermoplastic blends made from formulations of 20-71-9, 35-56-9 and 50-42-8 PE-SP-EG are much rougher, with less evenness indicating worse phase dispersion, and there are several cracks upon breaking. In contrast, the blending formulations of 65-27-8 and 80-13-7 PE-SP-EG seem to exhibit higher homogeneity in blending with less phase separation, suggesting that these two might be well desired formulations for good phase interaction and enhanced performance properties. Between these two formulations, 80-13-7 PE-SP-EG formulation has even better surface homogeneity as shown in the SEM image under the same scale.





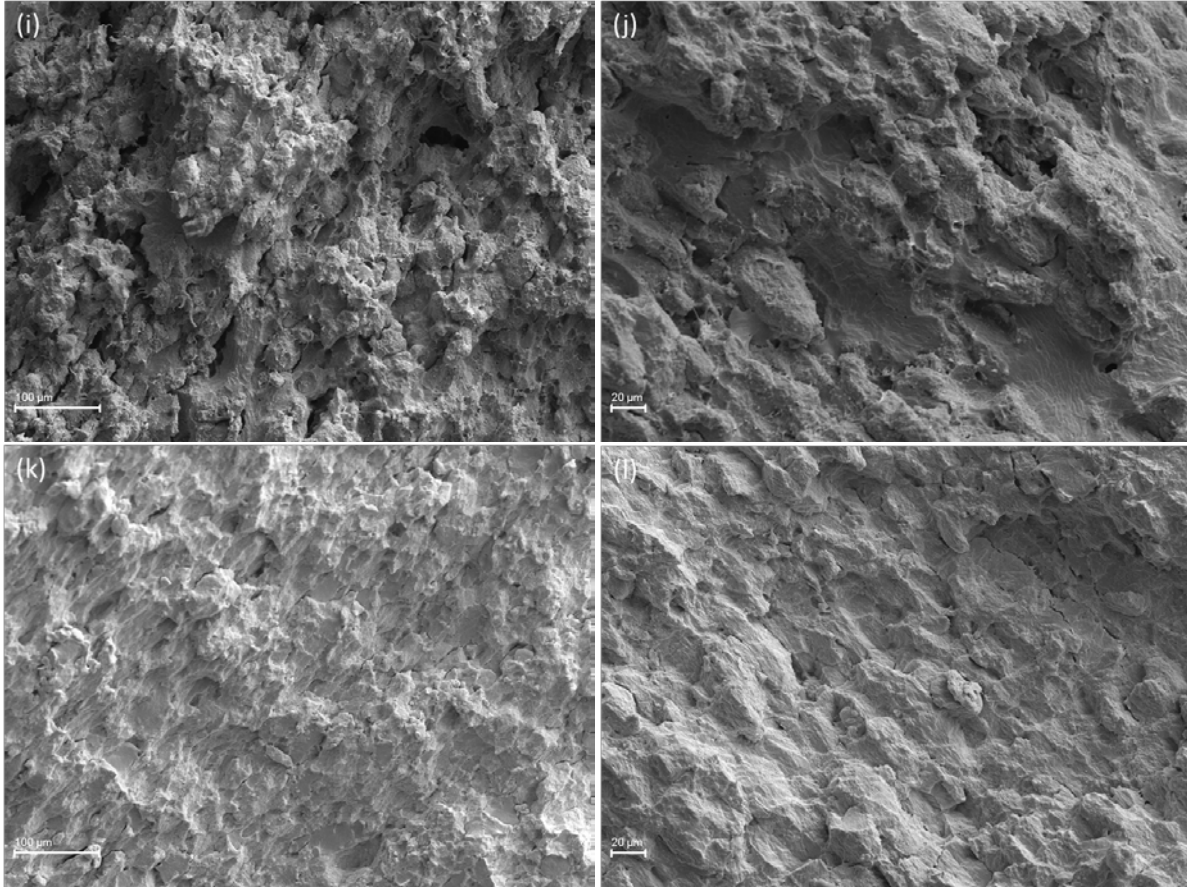


Figure 4.5 SEM of Spirulina Thermoplastic Blends

(a), (b) 20-71-9 PE-SP-EG; (c), (d) 35-56-9 PE-SP-EG; (e), (f) 50-42-8 PE-SP-EG; (g), (h) 65-27-8 PE-SP-EG; (i), (j) 80-13-7 PE-SP-EG; (k), (l) 100 PE.

Conclusion

TGA of the PE blended SP-EG biomass shows that the SP degrades at 225-375 °C with a peak centered at 300 °C, while the PE degrade at 480 °C. These peaks intensity are proportional to the contents of the two. DSC data of PE-SP-EG thermoplastic blends shows that 70 °C peak represents the SP denatures while the 130 °C one represents the PE melting point.

DMA of PE-SP-EG thermoplastic blends showed a shift of peak Tan Delta from lower temperature to a higher one, and meanwhile an increasing of modulus with the increasing percentage of polyethylene. Formulations that contain more than 50% SP has higher Tan Delta

values before 100 °C, while above 100 °C formulations have more than or equal to 50% PE shows higher Tan Delta. The 80% PE formulation have highest modulus values other than 100% PE and even higher Tan Delta values than PE, suggesting this is the best PE blended Spirulina bio-plastic formulation.

The tensile properties of PE-SP-EG thermoplastic blends shows that 80-13-7 PE-SP-EG is a better bio-plastic blend because it has better load and extension characteristics and better load-extension curve than all the other blended bio-plastics.

SEM fracture micrographs of PE-SP-EG thermoplastic blends shows that 80-13-7 PE-SP-EG seem to exhibit best homogeneity in blending with less phase separation, suggesting it is the most desired formulations for good phase interaction and enhanced performance properties.

CHAPTER 5

COMPATIBILIZATION OF SPIRULINA MICROALGAE BIOMASS

Experiments

DMA flexbars that has no compatibilizer which has formulations of 50-42-8 PE-SP-EG and that has compatibilizer which has formulations of 48-41-8-3 PE-SP-EG-CP were molded.

DMA, FT-IR and SEM were performed according to procedures in Chapter 1.

Results and Discussion

Dynamic Mechanical Analysis of Spirulina Compatibilization Bio-plastics

Figure 5.1 shows the DMA data of Spirulina compatibilized bio-plastics, and it suggests that there is no significant difference in modulus or Tan Delta between Spirulina blending bio-plastic with and without compatibilizer like Polyethylene-grafte-maleic anhydride (PE-g-MA) at a weight percentage of 3%.

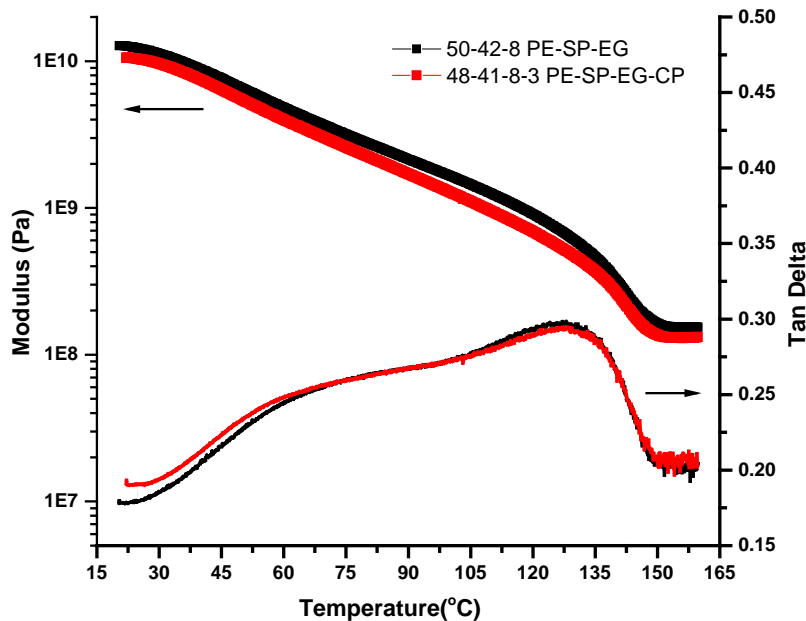


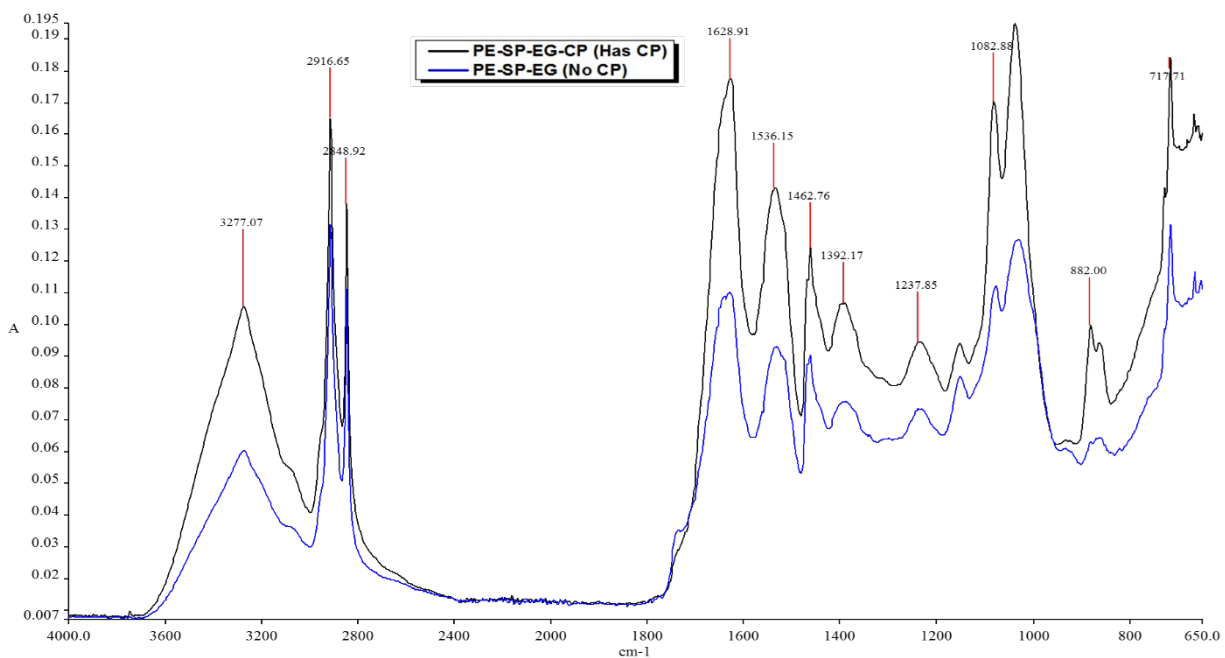
Figure 5.1 DMA of Flexbars of Spirulina Compatibilized with PE-g-MA

FT-IR of Compatibilized Spirulina Bio-plastics

Figure 5.2 shows the FT-IR of flexbars of Spirulina compatibilized with Polyethylene-grafted-maleic anhydride (PE-g-MA). FTIR investigation can be used as an effective tool to prove the structural changes in proteins. Particularly, the amide I band in the range between 1600 and 1700 cm^{-1} and amide II band in the region of 1510 and 1580 cm^{-1} provide useful information. Amide I is useful for the analysis of the protein secondary structure. It is also the most intense absorption band in proteins. With mainly affected by C=O stretching and a minor contribution from C–N stretching, the amide I band moves to higher wavenumber.

In the presence of PE-g-MA, this peak at 1640 was shifted to lower wavenumbers, located at around 1630 cm^{-1} . The shifts in amide I bands toward a lower frequency were indicative of increased amounts of ordered β -sheet structures [30]. The amide II band originates from the N–H bending and C–H stretching vibrations. Compared to amide I, the amide II is much less conformationally sensitive while much more sensitive to the environment of the N–H

group [31]. Therefore, the amide II band can be used to deduce changes to the environment of the N–H groups and respond to differences in hydrogen-bonding environments [32]. In general, stronger hydrogen-bonded N–H groups absorb at higher frequencies. Greater numbers of H bonding and thus more H bonded peptide groups was observed at higher wavenumbers. From figure 5.2, there seems no shifting to larger wavenumbers for the two bands at 1540 and 1517 with compatibilized was added. However, the intensity of the band for both of the bands seems to be larger, and this may indicate stronger H-bonding was formed with the presence of compatibilizer.



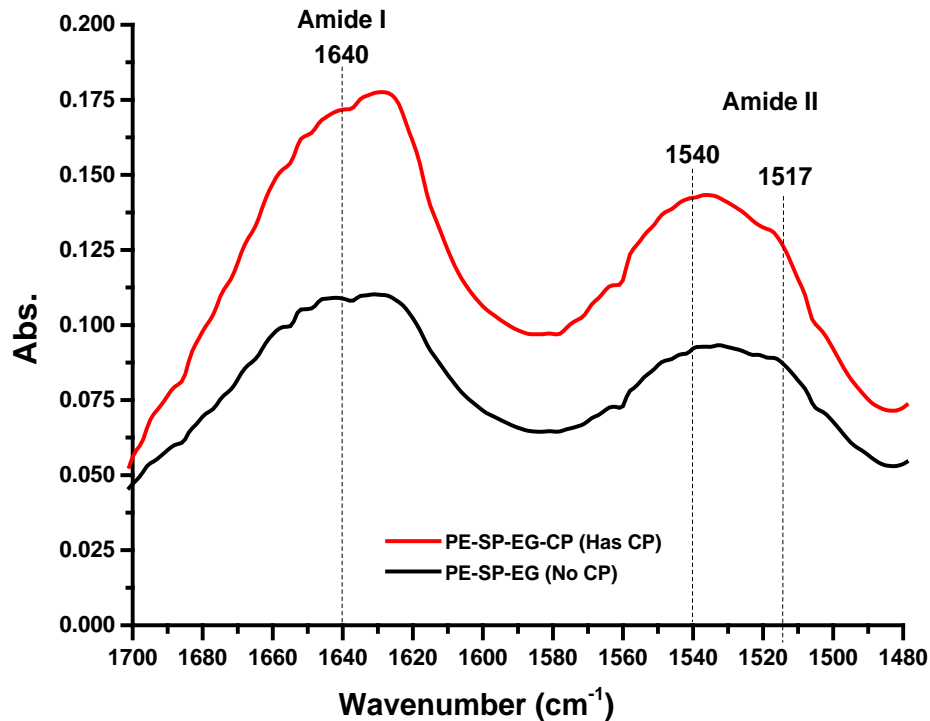


Figure 5.2 FT-IR of Flexbars of Spirulina Compatibilized with Polyethylene-grafted-maleic anhydride

SEM images of Compatibilized Spirulina Bio-plastics

Figure 5.3 shows the SEM images of the compatibilized Spirulina bio-plastics. There are no big difference between the sample has no compatibilizer and that with compatibilizer added. However, the sample that has compatibilizer seems to have more smooth fracture surfaces than the one has no compatibilizer within. This may indicate that the compatibilizer has some effect on ameliorating the compatibilization of the PE, the hydrophobic, and algae, the hydrophilic, phases in thermal blending plastics.

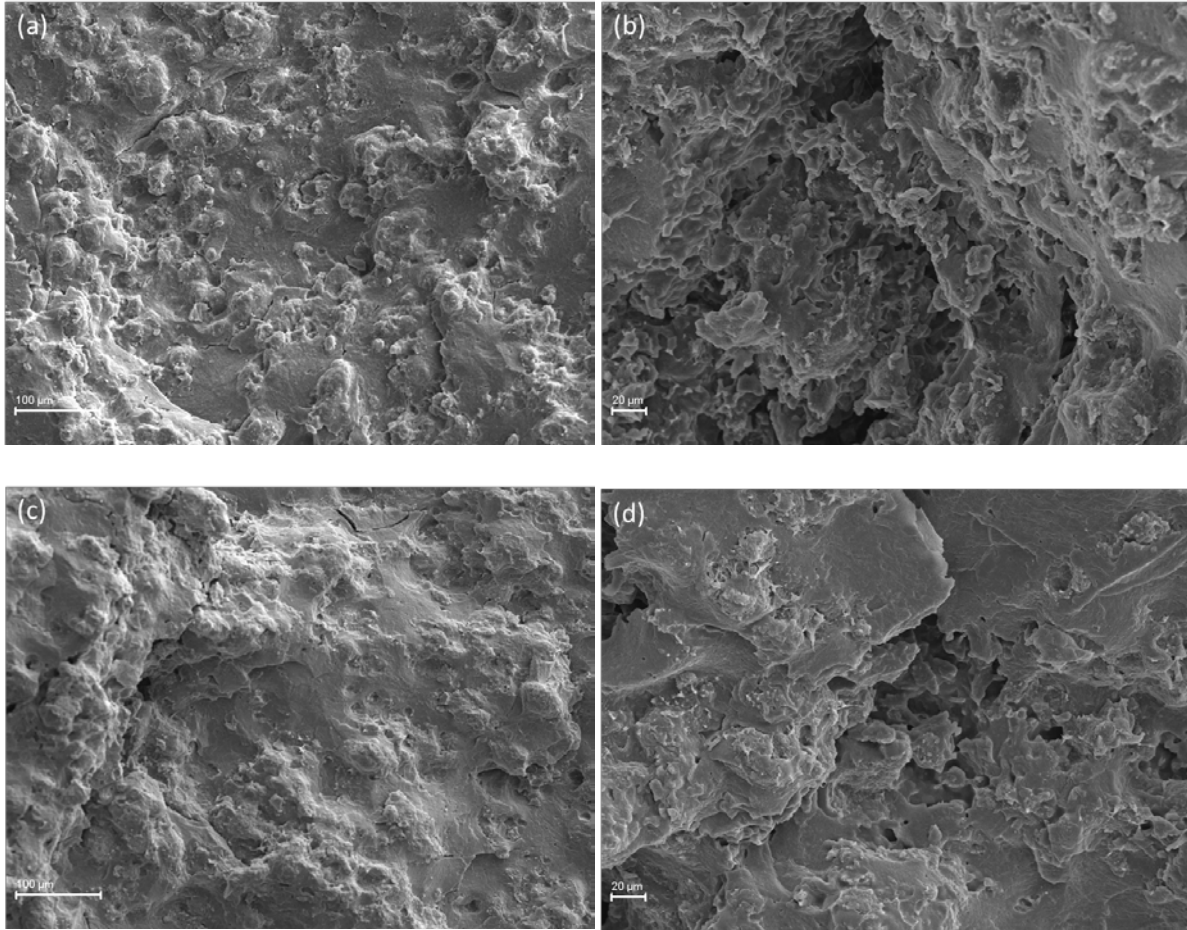


Figure 5.3 SEM of Spirulina Thermoplastic Blends with or without Compatibilizer

(a), (b) Thermoplastic With No Compatibilizer; (c), (d) Thermoplastic With Compatibilizer

Conclusion

A weight percentage of 3% compatibilizer as Polyethylene-grafted-maleic anhydride (PE-g-MA) has barely effect on the dynamic mechanical property of Spirulina bio-plastic. However, The FT-IR graph shows that in the presence of PE-g-MA, this peak at 1640 was shifted to lower wavenumbers, located at around 1630 cm^{-1} . The shifts in amide I bands toward a lower frequency were indicative of increased amounts of ordered β -sheet structures. Also, the intensity of the band for amide II bands seems to be larger, and this may indicate stronger H-bonding was formed with the presence of compatibilizer. From the SEM images we can see that

the sample that has compatibilizer seems to have more smooth fracture surfaces than the one has no compatibilizer within.

3% compatibilizer seems to have some effect on making the bio-plastic containing more ordered in secondary structure and increase H-bonding intensity and approves phases' compatibilization. However, the mechanical properties have no increase. This may be because of the small amount of the compatibilizer using.

CHAPTER 6

ODOR REMOVAL STUDY OF SPIRULINA BIO-PLASTIC

GC-MS Analysis of Activated Carbon Co-processed Spirulina Bio-plastics

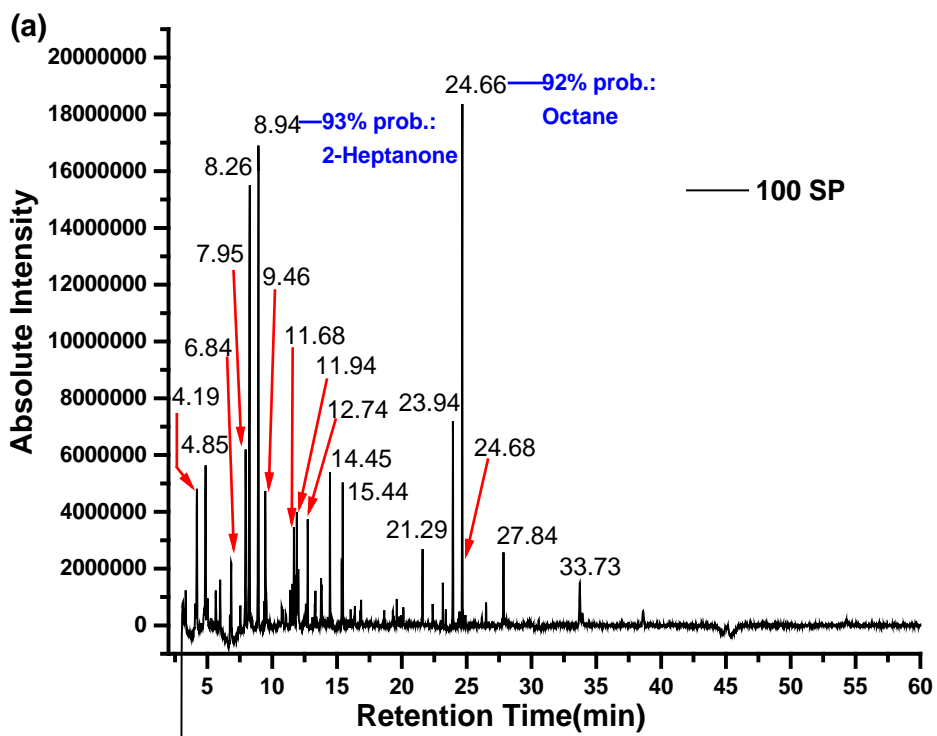
The GC-MS can help detect the volatile odor substances in the Spirulina biomass based bio-plastics. Flexbars of 100 Spirulina and 95-5 Spirulina-Activated Carbon were made. The headspace solid phase micro-extraction (SPME) method was used for the sampling. From each formulation, approximately 0.120 g piece was chopped and put into 2 ml head space vials sealed by septa and went through thermal equilibrium at 80 °C for two hours. After 2 minutes cooling, a 65 µm PDMS/DVB fibre within a SPME holder was inserted into the vial to extract volatiles for 5 minutes.

The GC/MS analysis was performed on a Shimadzu QP5000 using a ZB-5 capillary column (Phenomenex 30m x 0.25mm x 0.25µm) with helium as the carrier gas. A glass inlet liner of 0.75 mm was installed for use in the injection port. The injection temperature and interface temperature were set to 250°C and 230°C, respectively. After the SPME fibre was injected into the GC/MS, desorption occurred for 2 minutes. A splitless injection was utilized at pressure of 15 psi. The oven was held at 40°C for 5 minute, ramped at 5°C/min to 200°C, and held at 200°C for 15 minutes. The mass spectrometric detector's scan mode was read at range of 50-300 m/z. The gas chromatograph peaks were integrated using the parameters of slope = 25,000/min and width = 3 sec. An NIST 98 library was used to identify the peaks.

Figure 6.1 shows the GC/MS results for the bio-plastic samples of 100 Spirulina and 95/5 Spirulina/carbon. Comparing the two graphs, the number and intensity of the peaks which have

retention time of 8.5 to 25 minute become less with activated carbon added. The number of peaks that have retention time of 6 to 9 minute seems remain the same but with intensity decreases for 95-5 SP-C sample. Numbers of peaks elute near 3 and 4 minutes decreased, but the intensity increased. It suggests that 95-5 SP-C have less volatile compounds than the 100 SP bio-plastic meaning that activated carbon has effect on absorbing volatile compounds and ameliorate the odorous features.

Algae's characteristic odor consists of many different compounds. Each compound contributes to the complexity of algae's odor. The GC/MS data indicates a pattern in the kinds of functional groups appearing. Some simple alkanes and ketones were found attributing the odor in green algae using Solid Phase Microextraction Gas Chromatography Mass Spectrometry [33].



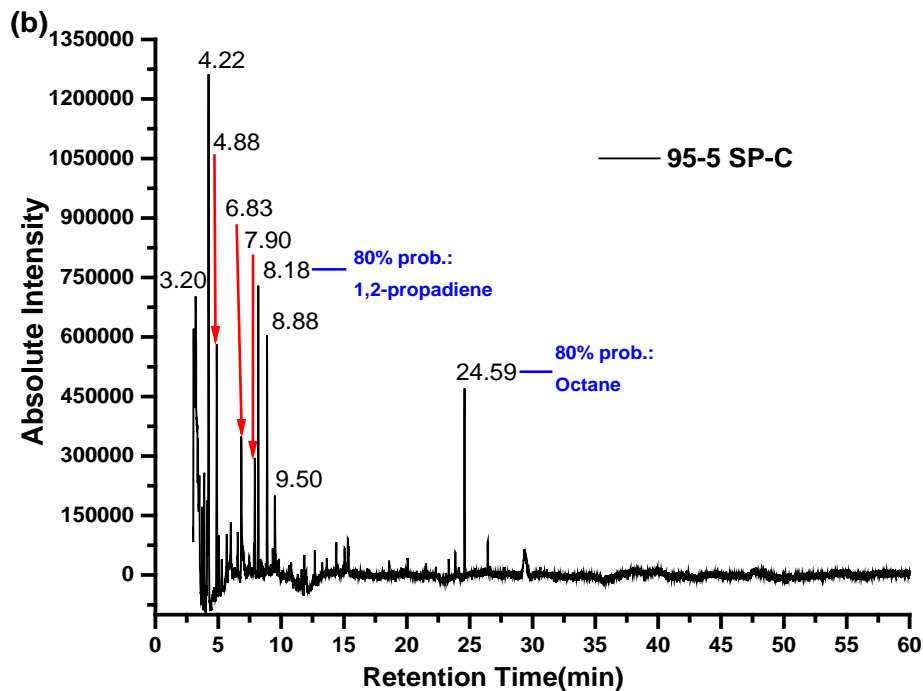


Figure 6.1 GC/MS Results of 100% Spirulina (a) and 95-5 Spirulina-Carbon(b) Bio-plastics

Conclusions

The GC/MS testing for the 95-5 SP-C bio-plastic were carried out by comparing with that of 100% Spirulina, and the results showed that activated carbon has effect on absorbing volatile compounds and ameliorate the odorous features, which was supported by the changes in the number and intensity of the peaks of their GC-MS spectrum.

CHAPTER 7

PROTEIN EXTRACTION OF SPIRULINA MICROALGAE AND ITS PLASTICS

Why Extract Protein in This Research

The compositional analysis shows results from several kinds of algae. Of the microalgae, Solix Microalgae is a nannochloropsis species which are mostly known from the marine environment [18]. GO2 and AlgaEvolve are green algae consortiums which are mixed species cultures. For macroalgae, we present compositions of Ireland Seaweed (green algae) Ulva (green algae) and Fucus (brown algae) both from Cape Cod, Massachusetts; and Gracilaria (red algae) from Waquoit Bay, Massachusetts. The specific composition analysis results of these algae are listed in Table 2.

From the analysis, it is found that, in general, the microalgae contain less moisture and ash in comparison to the macroalgae. Also, it seems microalgae have higher protein contents than macroalgae. The protein content of algae is of importance to the development of bio-plastics and thermoplastic blends.

Table 2 Composition Analysis of Algae

%	Moisture	Volatiles	Ash	Fixed Carbon	Crude Protein	Carbohydrate	Lipids	Other
Micro-algae	Solix	3.8	73.8	9.2	13.2	33.9	19.5	37.6
	GO2	8.3	63.5	20.3	8.0	30.1	7.5	60.7
	Alga Evolve	2.7	65.3	19.3	12.7	33.8	33.1	32.2
Macro-algae	Ireland	8.6	60.0	22.2	9.2	8.2	26.2	64.5
	Ulva	16.9	60.7	19.9	2.6	9.6	56.5	32.3
	Fucus	15.9	63.4	17.1	3.6	8.5	36.5	49.9
	Gracilaria	8.4	47.4	33.1	11.2	9.9	40.0	48.7

Figure 7.1 shows the microalgae plastics sample surfaces. Comparing the surfaces of the 100% bio-plastics made from the three microalgae whose crude protein contents are similar, it is most probably the high content of lipids of Solix that leads to great cracks on its surface while the surfaces of GO2 algae and AlgaEvolve remain smooth. This stays true with both 10 and 20% water or glycerol as plasticizer. Comparing the surfaces of bio-plastics made of GO2 algae and AlgaEvolve containing 20% of glycerol or water in the formula, fissures occur in those of AlgaEvolve. This is most probably due to the higher content of carbohydrate of AlgaEvolve than GO2 Algae. This again proves that protein is the most important component in making good bio-plastics. Since excluding lipids and carbohydrates are more time-consuming, protein extraction was the best way to get the most useful part of the algae that contribute to making bio-plastics.

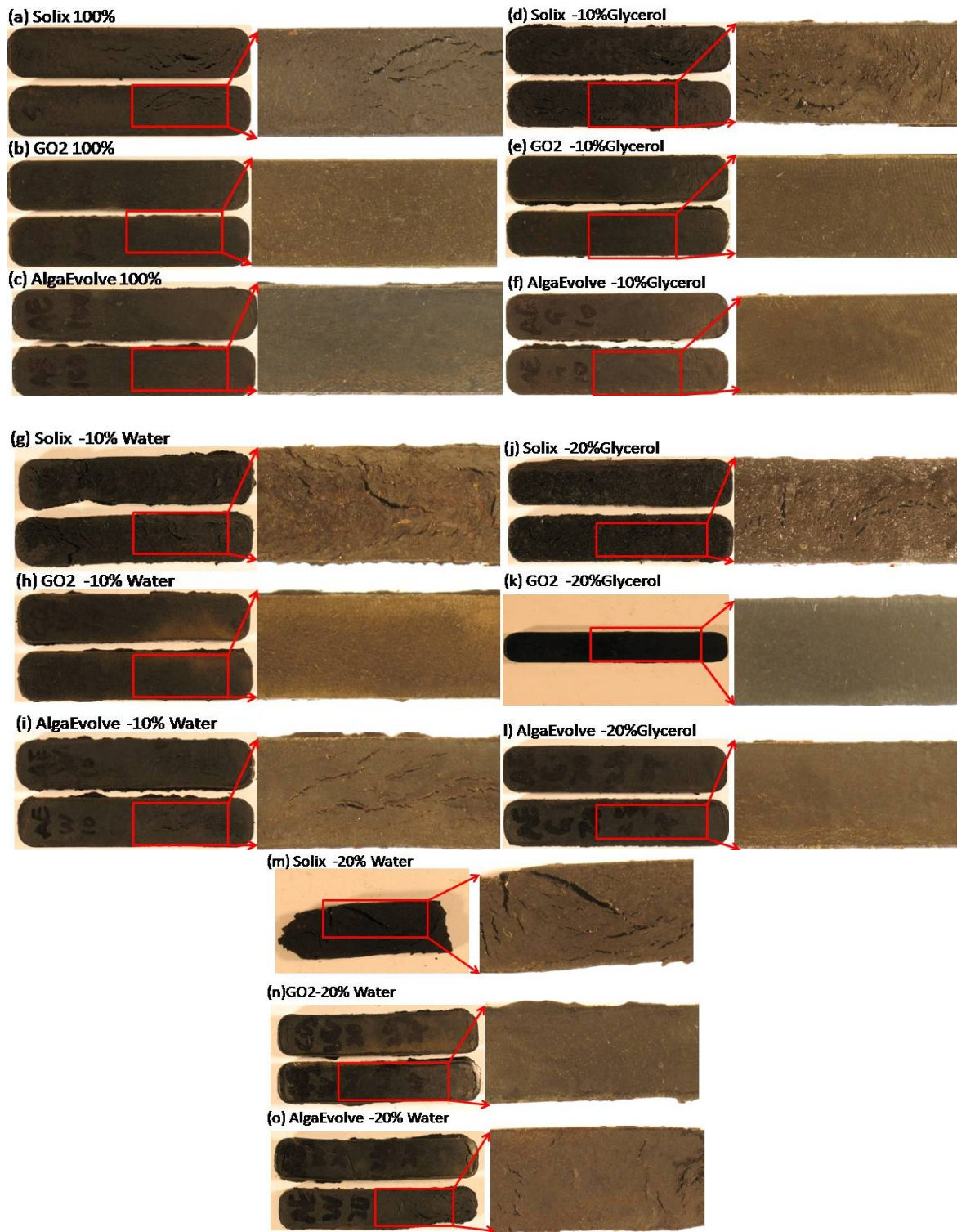


Figure 7.1 Surface Conditions of Bio-plastics Made from Microalgae and Plasticized with Glycerol and Water

Protein Extraction Protocol

The *Spirulina* microalgae samples were received from ALGIX Company, and the corresponding power was obtained by grinding the dry material with a pestle and a mortar. 10 g of this powder was used for protein extraction.

Dry material was suspended in deionized water and gently stirred for 12h at 4 °C. Afterward, it is ground using a blender for 5 min at 4 °C. The suspension was centrifuged at 15,000×g for 20min and the supernatant was collected and kept at 4 °C. The pellet was treated with NaOH (0.1M) in the presence of mercaptoethanol (0.5% v/v) and the mixture was gently stirred for 1h at room temperature. Then the treated pellet mixture solution was centrifugation at 15, 000×g for 20min and supernatant was collected. The supernatant was combined with those from the discard pellets. For precipitation of protein, cold 25% TCA (TCA: homogenate=2.5:1, v/v) was added and kept in ice bath for 30 min. The solution was centrifuged (15,000g, 20min) at 4 °C, the supernatant was discarded. The pellet was rinsed with cold 10% TCA, centrifuge (15,000g, 2min) at 4 °C and discarded the supernatant. Solubilization of the protein was done by adding 5% TCA (5:1) to the pellet and centrifuging at 15,000g for 20 min at 20 °C. The supernatant was discarded. The pellet was used for further experiments.

The micrographs of the original *Spirulina* powder and the final pellet are shown in Figure 7.2 and Figure 7.3. From Figure 7.2, *Spirulina* cells can be clearly seen. The *Spirulina* powder has the algal cells. *Spirulina* cells appear as a mass of intertwined unicellular spiral filaments, or trichomes under the microscope. Each is of variable length (typically 100–200 microns) and with a diameter close to 8–10 microns [34]. From the microscope image, the spiral filaments may have further intertwined into circle and forming a sphere. The cells may be cut to shorter curled filaments and distorted to various 3D shapes. That may be because the cells undergone

mechanical stresses when the material was ground to fine powder. Figure 7.2 shows the microscopic image of the protein pellet extracted from Spirulina powder. In general, the cells are ruptured into various-shaped transparent appearance indicating the cells were broken to release protein due to TCA precipitation. Though the broken cells are still stay in the pellets since the pure protein was not be observed and the broken cell may also have protein, TCA precipitation of the protein successfully broke many cells and liberated free protein.

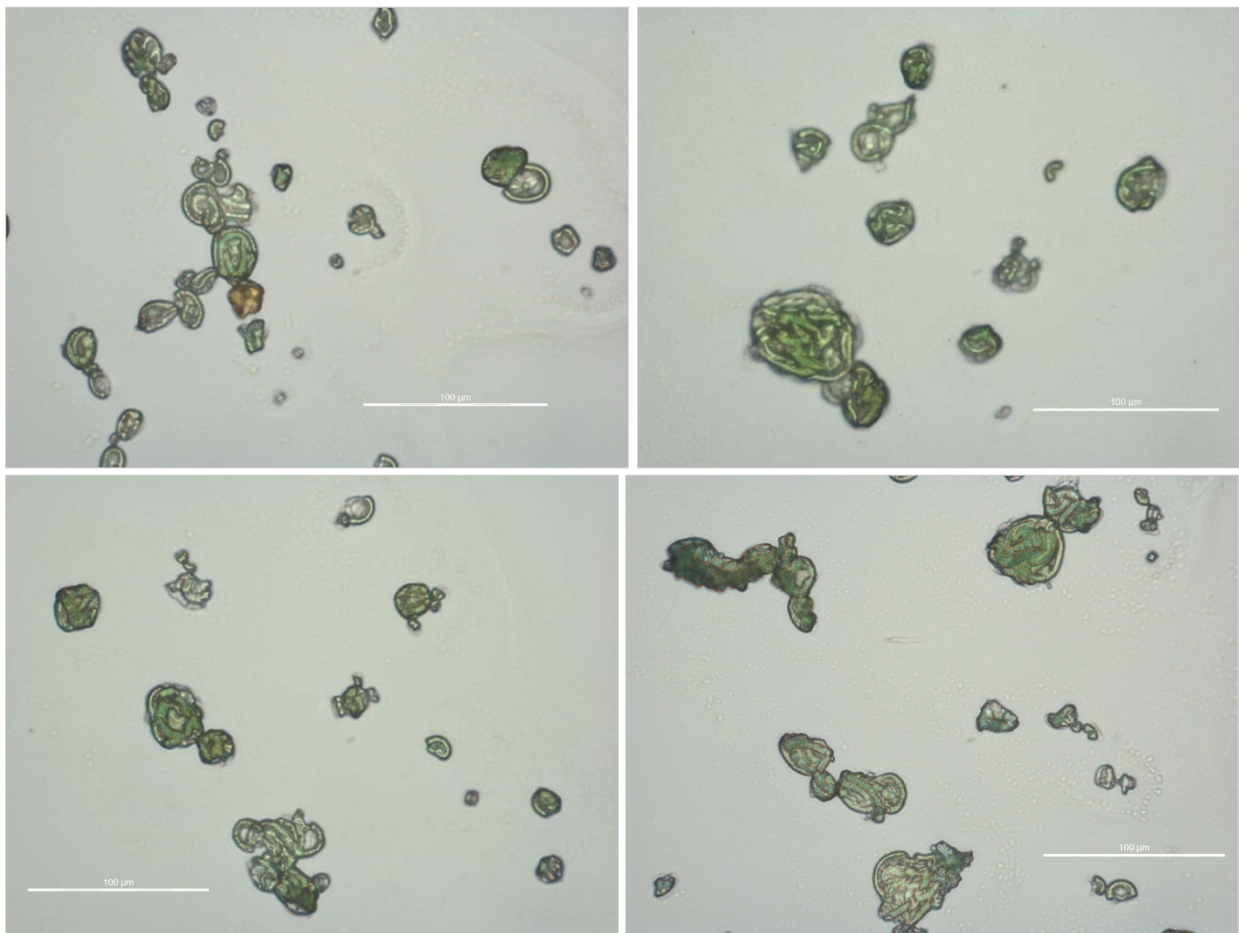


Figure 7.2 Microscopic Images of Spirulina Powder (scale bar: 100 μm)

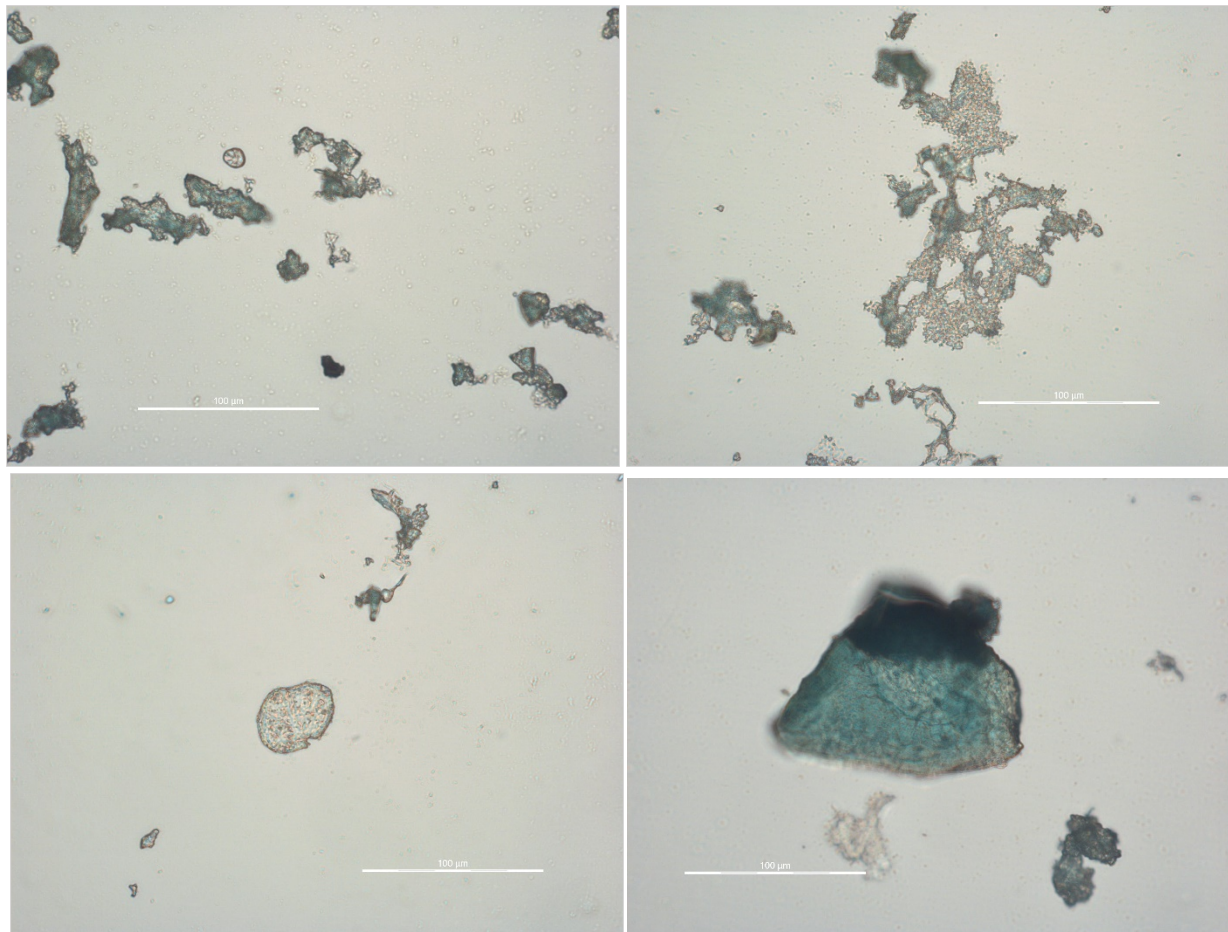


Figure 7.3 Microscopic Images of Spirulina Protein Pellets (scale bar: 100 µm)

Bicinchoninic Acid (BCA) Protein Assays

The bicinchoninic acid protein assay was done following the directions of Thermal Scientific BCA Protein Assay Reagent guidebook. Five weighed protein pellet samples were dissolved in SDS solution (5% w/v in 0.1N NaOH) and pH=7 buffer added to these to various extents. For the pellet samples numbered 6, 7, to 10, they are diluted with buffer to 12, 8, 5, 2, 1 (no dilution), respectively. Bovine serum albumin (BSA) solution was employed as a protein standard of 200, 400, 800, 1000 µg/ml to produce a standard curve. A pH=7 buffer was used as a control with those samples. After the samples were treated with BCA working reagent and vortexing, the samples were incubated at 60 °C for 15 min. The absorbances at 562 nm

wavelength were measured. The absorbance of BSA solutions of known concentration determined the standard curve and the absorbances of Spirulina protein samples were used to find the corresponding protein contents using the standard curve. Then dilution factors were taken into account to get the protein concentration. The average of the 5 results was obtained as the protein concentration of the protein pellet. BCA assay standard curve for protein content prediction is shown in Figure 7.4 and the corresponding predicted protein content is listed in Table 3.

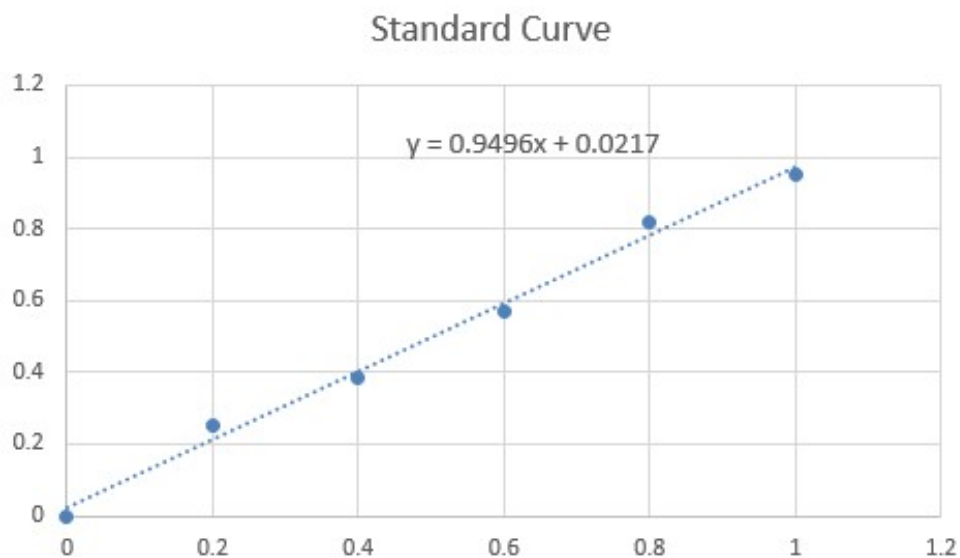


Figure 7.4 BCA Assay Standard Curve for Protein Content Prediction

Table 3. BCA Assay Predicated Protein Content

	Sample #	Concentration (mg/ml)	Dilute factor	Absorbance at 562nm	Predict con.of individual sample(mg/ml)	Protein con.(mg/ml)	average protein con.(mg/ml)
Buffer	11	0		0			
BSA	1	0.2		0.254	0.2		
	2	0.4		0.382	0.4		
	3	0.6		0.572	0.6		
	4	0.8		0.818	0.8		
	5	1.0		0.953	1.0		
Protein pellet	6	0.83	12	0.156	0.141	1.697	1.420
	7	1.25	8	0.185	0.172	1.376	
	8	2.00	5	0.302	0.295	1.476	
	9	5.00	2	0.620	0.630	1.260	
	10	10.0	1	1.25	1.293	1.293	

Since 1ml solution contains 10mg pellet (1.42mg protein), so the pellet contains $1.42/10*100\%=14.2\%$ protein. And 0.40g of protein pellet was also put into oven at 70 °C for 72 hours and was weighed again (0.10g) to get the possible water amount of $(0.4-0.1)/0.4*100\%=75\%$.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was done using the Laemmli system. The acrylamide (Biorad) concentrations were 5% for the stacking gel and 10% for the resolving gel. The protein extracts and a low molecular weight pre-stained standard were allowed to migrate for 40 min at 200 V. Coomassie blue staining and fixation was then performed to get a general idea of the molecular weight of the proteins. Two gels were made and both the gels have the standard loading at the first and last wells. 3 samples were loaded in wells between those of the standards. The standards laded were were 5µl each, and the 6 samples were 5µl each, containing 5, 10, 15, 20, 25, and 30 µg, respectively. The pre-stained standards had 6 proteins of molecular weight which are listed in table 4. Figure 7.5 shows the SDS-PAGE gel running result of only the second gel, which has

samples containing 20, 25, 30 μg in 5 μl . Compared to the proteins contained in pre-stained standard, the two proteins that have lowest molecular weight of those proteins may have run down out from the gel. The protein samples were stained continuous blue meaning the Spirulina protein has miscellaneous proteins ranging from about 47 kDa to well above 103 kDa. The two major groups of protein are about 50 kDa and 120 kDa.

Table 4. Protein Contained in Pre-Stained Standards

kDa	Protein
103.7	Phosphorylase B
81.1	Bovine serum albumin
47.7	Ovalbumin Carbonic
35.8	Carbonic anhydrase
27.1	Soybean trypsin inhibitor
19.3	Lysozyme

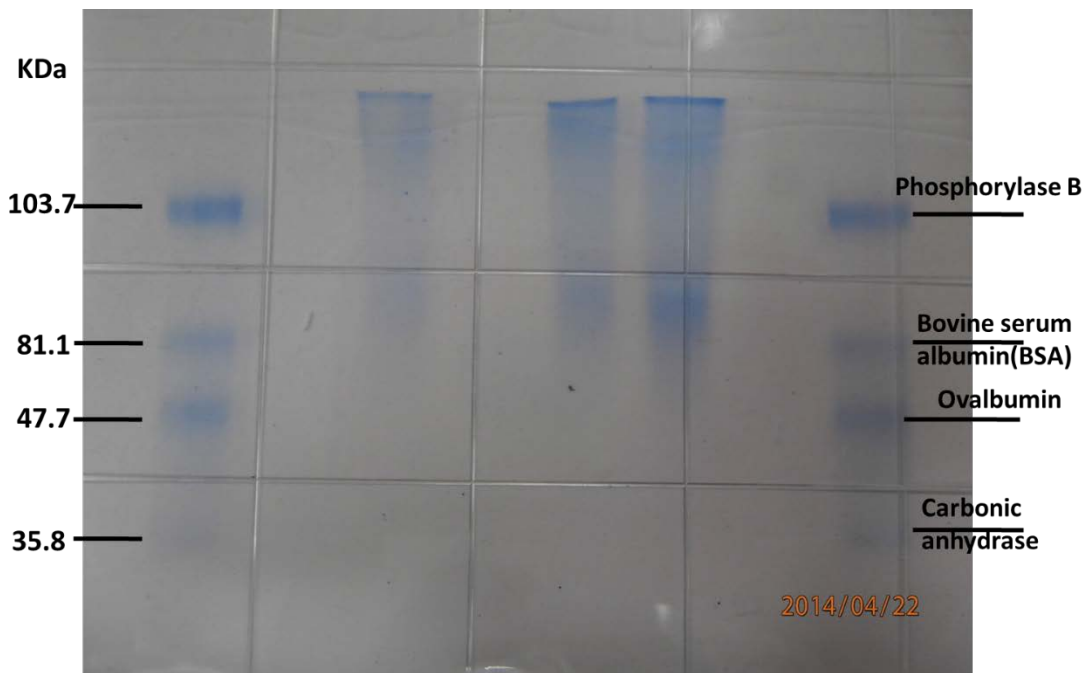


Figure 7.5 Spirulina SDS-PAGE Results

Thermal and Dynamic Mechanical Analysis Spirulina Protein Bio-plastics

Figure 7.6 shows the TGA of Spirulina dry powder and its extracted protein pellets under three different conditions. The dried protein pellets were obtained by drying at 40 °C and 70 °C for 72 hours. The Spirulina dry powder degraded at around 325 °C, which is also the degradation point for its corresponding protein regardless of its conditions. Three degradation steps occurred for the wet protein pellet. The first one at about 75 °C is due to vaporizing of absorbed water and the second one at around 130 °C is due to the loss of bound water. Finally, the small, broad peak at 325 °C represent protein degradation. After drying at 40 °C for 72 hours, the wet protein pellet lost nearly all of the directly absorbed water, but this normal drying procedure seemed to have no effect on expelling the bound water, so that the peak at around 75 °C has disappeared while that of about 130 °C still can be observed on the TGA curve of the 40 °C dried protein pellet. For the 70 °C dried protein pellet, only the peak at 325 °C was left, meaning this protein was nearly dry enough.

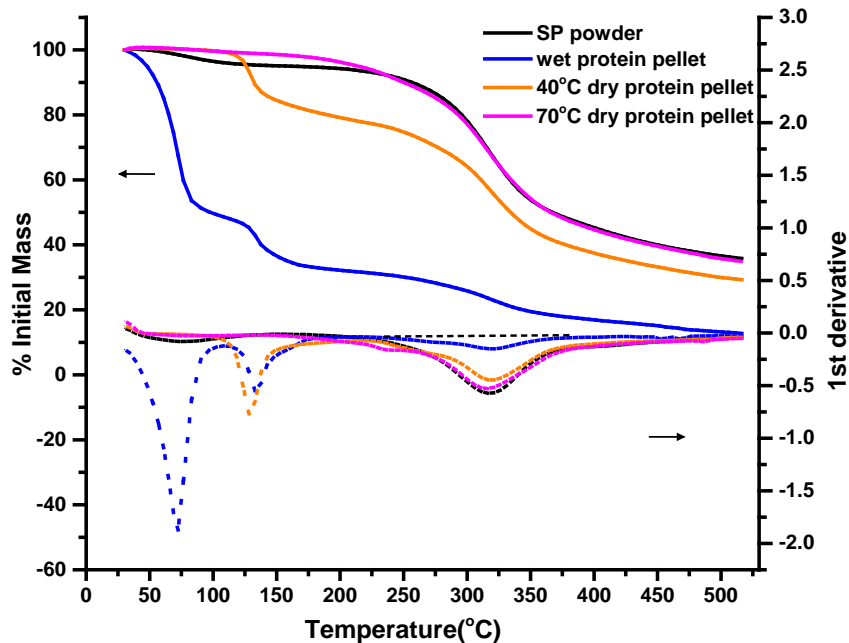
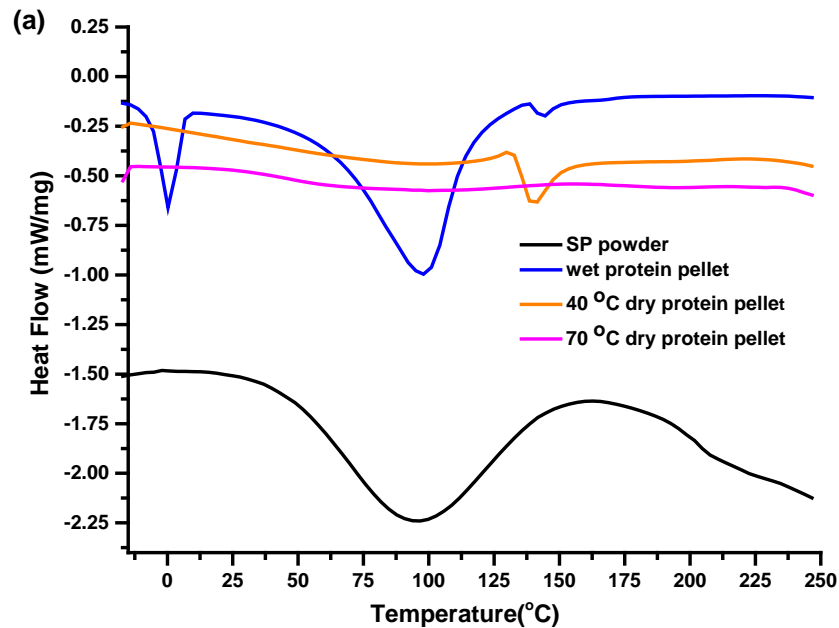


Figure 7.6 TGA of Flexbars of Spirulina Extracted Protein Pellet

Figure 7.7 shows the DSC data of flexbars of Spirulina dry powder and its extracted protein pellets under three different hydration conditions. As can be seen in Figure 7.7, there are three endothermic peaks in wet protein pellet. The peak at 0 °C represents the evaporation of free water, while the peak at around 100 °C and 130 °C represents denaturation of Spirulina components. The peak at about 130 °C could be the denaturation temperature of the protein especially. For the 40 °C dried protein pellet, two peaks around 100 °C and 130 °C could still be observed from its DSC curve. However, for the 70 °C dried protein pellet, just the protein gradually denaturation step starting at 30 °C and ending at 150 °C was left. This temperature range was informative for making bio-plastics using these extracted protein pellet. The processing temperature for making bio-plastic with protein should be set at 150 °C where protein maximally denatured.



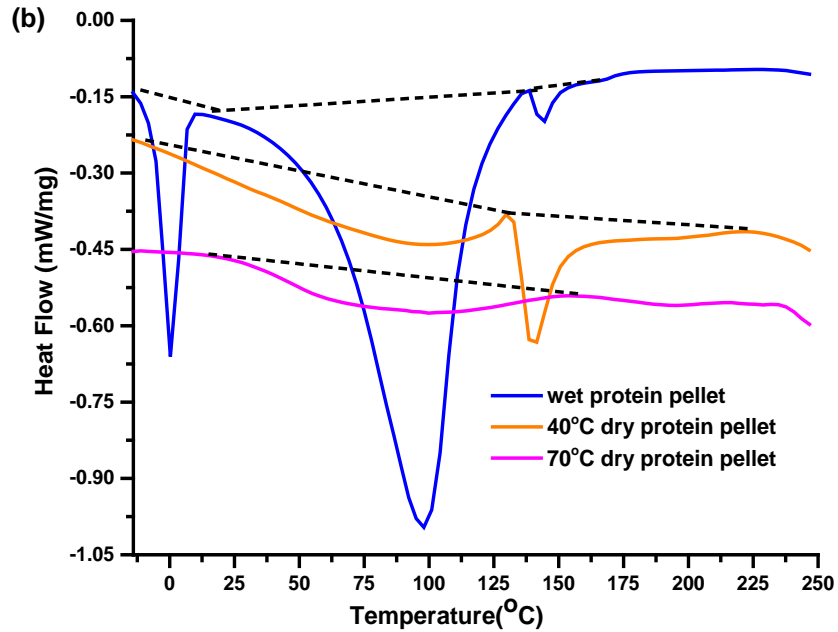


Figure 7.7 DSC of Spirulina Extracted Protein Pellet

DMA flexbars of extracted protein pellet, which was dried at 40 °C for 3 days, were thermal compression molded at 150 °C and its DMA result was compared with that of 100% Spirulina dry powder as shown in Figure 7.8. For the DMA of extracted protein, the initial modulus of bio-plastics made from 100% extracted protein at the temperature range from 25 °C to 50 °C was a little lower than that of the 100% Spirulina dry powder, and so did the Tan Delta. But in the temperature range from 50 °C to 160 °C, there is a reverse trend on the modulus comparison between 100% extracted protein and 100% Spirulina dry powder, but the Tan Delta of bio-plastic made from 100 % extracted protein was much lower than that of 100% Spirulina dry powder. Considering the high modulus through all the temperature raising range, and the minute glass transition phase that occur very early as 40-80 °C of the 100% protein pellet, it behaves more as a stiff material rather than a plastic.

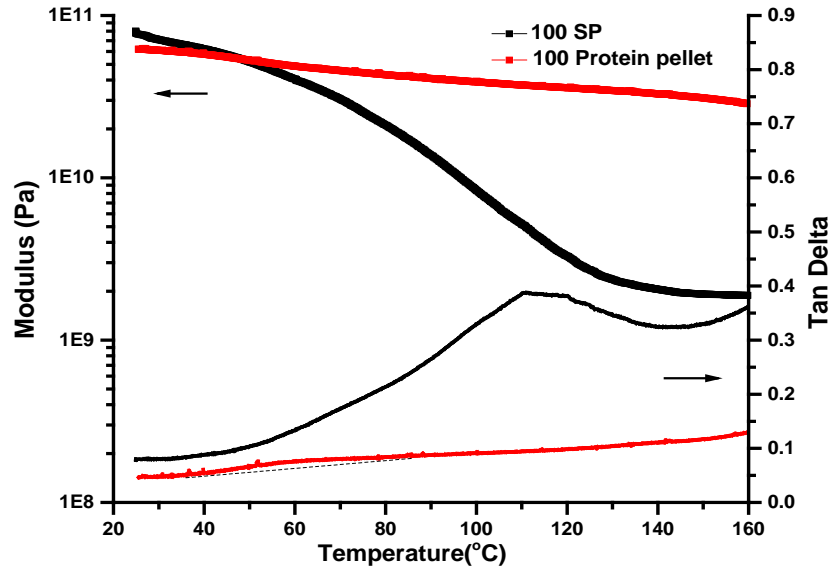


Figure 7.8 DMA of Spirulina Extracted Protein Bioplastic

Figure 7.9 shows when the protein pellet was blended with 80% PE and plasticized with 7% EG, the T_g stays the same the blended Spirulina plastic. The protein pellet blended plastic has higher modulus and lower Tan Delta values than that of blended Spirulina sample through almost all the temperature range, meaning it has a good potential of developing bio-plastics but is stiffer.

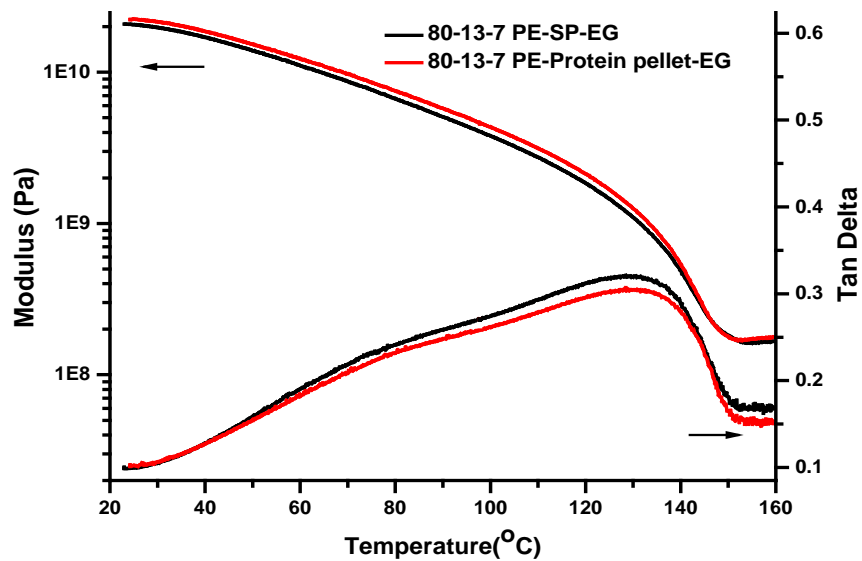


Figure 7.9 DMA of PE-Blended Spirulina Extracted Protein

Examinations of the subjective appearance and surface morphology were carried out on flexbars made of Spirulina powder and extracted protein after DMA testing was applied as shown in Figure 7.10. Dented and bulged surfaces were formed on the surface of the flexbar made of Spirulina powder was caused by the clamping heads of DMA as shown in red box in Figure 7.10, but these didn't occur on the flexbar made of Spirulina extracted protein. Incidentally, the color of the Spirulina extracted protein flexbar was black and is darker than that of flexbar of Spirulina powder, which is dark brown perceived by human eyes under normal indoor light conditions.

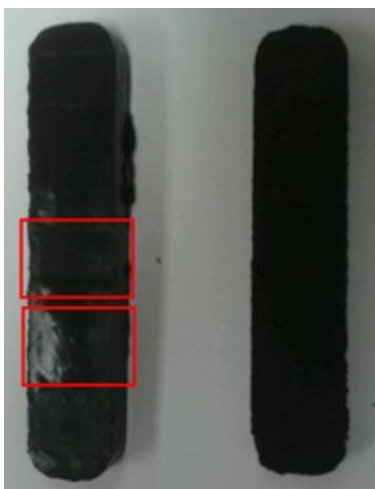


Figure 7.10 Surface Morphology of Spirulina (left) and Spirulina (Right) Extracted Protein Flexbars after DMA Testing

Conclusions

The pellet and protein extracted from Spirulina was done by using a typical protocol, and the thermal, mechanical and morphology of the bio-plastics made of this pellet was explored. The optical observation of Spirulina powder and its pellets showed that trichloroacetic acid (TCA) precipitation of the protein successfully broke the cell and obtained free protein. According to the BCA assay, the protein content of the protein pellet obtained is 14.2% and

SDS-PAGE testing indicated that Spirulina has miscellaneous proteins than ranging from about 47 kDa to well above 103 kDa. The two major groups of protein are about 50 kDa and 120 kDa. The TGA of Spirulina Extracted Protein bio-plastics demonstrated that the extracted protein pellets has the same degradation point as that of Spirulina dry powder, while there are directed and bounded water in the wet Spirulina pellets and the dried one at different temperature depends on the moisture. The DSC implied that just one main protein gradually denaturation step starting at 30 °C and ending at 150 °C was left, which suggested that the processing temperature making bio-plastic with extracted protein pellet can be set at 150 °C. The DMA indicated that in the temperature range from 50 °C to 160 °C, the modulus of bio-plastics made of 100% extracted protein were higher than that of 100% Spirulina dry powder, but the Tan Delta of 100% extracted protein was much lower than that of 100% Spirulina dry powder.

CHAPTER 8

CONCLUSIONS

The basic properties, such as thermal, mechanical and morphology characteristics, of several different kinds of algae were investigated for its potential use in making bio-plastics, especially that of Spirulina and that of its extracted protein pellets. And some key conclusions were obtained as the followings.

As to the micro- and macroalgae, proximate constituent analysis of the raw material showed that the microalgae contain more fixed carbon and protein than the macroalgae. DMA of flexbars of Ireland Seaweed blended with water showed a substantial effect from plasticization with water. Flexbars made of Ireland Seaweed have higher modulus which means more stiffness, while those made of Solix Microalgae show higher Tan Delta which means better energy absorption property.

When it comes to the plasticized Spirulina bio-plastics, different weight ratio of Ethylene Glycol plasticized Spirulina bio-plastics were made and evaluated by thermal, mechanical and morphological properties. The TGA results of the Spirulina bio-plastics shows the degradation peak at about 310 °C consistent well with that of its dry powder, and the adding plasticizer Ethylene Glycol aided the degradation and allowed its degradation starting at a lower temperature, which resulted from the interaction between carbohydrates and protein molecules was interfered by Ethylene Glycol. The DSC data show two endothermic peak. For the 5%, 10%, 15% EG formulation, the SP denaturation peak at around 75 °C is left shifted than that of the dry Spirulina powder as indicated in Figure 3.2, meaning a plasticization effect of EG which would

lost with EG% more than 20%. The 125-200 °C peak indicates that EG would evaporate from the bio-plastics from 125-200 °C if the EG% is higher than 10%. DAM shows that with the increase of EG, the bio-plastic become less stiff and has lower Tg. However, for the 75-25 and 70-30 SP-EG, the transition from glassy to rubbery region are too broad and happen at room temperature making them unacceptable for end use. The 95-5, 90-10, and 85-15 SP-EG formulation have good plasticizing effect, relatively moderate modulus and tan delta and among which 90-10 has considered the best balance of modulus and flexibility. The Instron tensile testing showed that 95-5 SP-EG bio-plastic has better load and extension but the Instron this may need further experiments to justify due to the inconsistency of the results.

When comes to the UHMW-PE blending Spirulina bio-plastics experiment, different weight ratio of PE with EG plasticized Spirulina biomass were made and evaluated by thermal, mechanical and morphological properties. The fracture surfaces images from SEM indicate that bio-plastic of 90-10 SP-EG formula shows no obvious cracks on the surface and its good morphological consistency indicating it has a well phase dispersion between Spirulina and the plasticizer and higher inter-surface adhesion. TGA of the PE blended SP-EG biomass shows that the SP degrades at 225-375 °C with a peak centered at 300 °C, while the PE degrade at 480 °C. These peaks intensity are proportional to the contents of the two. DSC data of PE-SP-EG thermoplastic blends shows that 70 °C peak represents the SP denatures while the 130 °C one represents the PE melting point. DMA of PE-SP-EG thermoplastic blends show a shift of peak Tan Delta from lower temperature to a higher one, and meanwhile an increasing of modulus with the increasing percentage of polyethylene. Formulations that contain more than 50% SP has higher Tan Delta values before 100 °C, while above 100 °C formulations have more than or equal to 50% PE shows higher Tan Delta. The 80% PE formulation have highest modulus values other

than 100% PE and even higher Tan Delta values than PE, suggesting this is the best PE blended Spirulina bio-plastic formulation. The tensile properties of PE-SP-EG thermoplastic blends shows that 80-13-7 PE-SP-EG is a better bio-plastic blend because it has better load and extension characteristics and better load-extension curve than all the other blended bio-plastics. SEM fracture micrographs of PE-SP-EG thermoplastic blends shows that 80-13-7 PE-SP-EG seem to exhibit best homogeneity in blending with less phase separation, suggesting it is the most desired formulations for good phase interaction and enhanced performance properties.

For the compatibilization effect of Polyethylene-graft-maleic anhydride (PE-g-MA) to the Spirulina bio-plastics, 3% PE-g-MA was added. 3% compatibilizer seems to have some effect on making the bio-plastic containing more ordered in secondary structure and increase H-bonding intensity and approves phases' compatibilization. However, the mechanical properties have no increase. This may be because of the small amount of the compatibilizer using.

The GC/MS testing for the 95-5 SP-C bio-plastic were carried out by comparing with that of 100% Spirulina, and the results showed that activated carbon has effect on absorbing volatile compounds and ameliorate the odorous features, which was supported by the changes in the number and intensity of the peaks of their GC-MS spectrum.

The pellet and protein extracted from Spirulina was done by using a typical protocol, and the thermal, mechanical and morphology of the bio-plastics made of this pellet was explored. The optical observation of Spirulina powder and its pellets showed that trichloroacetic acid (TCA) precipitation of the protein successfully broke the cell and obtained free protein. According to the BCA assay, the protein content of the protein pellet obtained is 14.2% and SDS-PAGE testing indicated that Spirulina has miscellaneous proteins than ranging from about 47 kDa to well above 103 kDa. The two major groups of protein are about 50 kDa and 120 kDa.

The TGA of Spirulina Extracted Protein bio-plastics demonstrated that the extracted protein pellets has the same degradation point as that of Spirulina dry powder, while there are directed and bounded water in the wet Spirulina pellets and the dried one at different temperature depends on the moisture. The DSC implied that just one main protein gradually denaturation step starting at 30 °C and ending at 150 °C was left, which suggested that the processing temperature making bio-plastic with extracted protein pellet should be setted at 150 °C. The DMA indicated that in the temperature range from 50 °C to 160 °C, the modulus of bio-plastics made of 100% extracted protein were higher than that of 100% Spirulina dry powder, but the Tan Delta of 100% extracted protein was much lower than that of 100% Spirulina dry powder.

Overall, Algae, especially microalgae have good potential of developing bio-plastics. The pure Spirulina bio-plastic has high modulus and Tan Delta. The 90-10 SP-EG was decided as best best EG plasticized Spirulina bio-plastic formulation. 80-13-7 PE-SP-EG was determined the the UHMW-PE blended SP/EG biomass thermoplastic. 3% PE-g-MA turned to have some effect on compatibilization of the PE blended thermoplastic but no notable elimination on its mechanical properties. 5% activated carbon showed to be effective in removing odorous compounds present in Spirulina bio-plastic. Spirulina has a miscellaneous proteins ranging from about 47 kDa to well above 103 kDa. The extracted protein exhibited good bio-plastic prospective, the processing temperature can be set at 150 °C.

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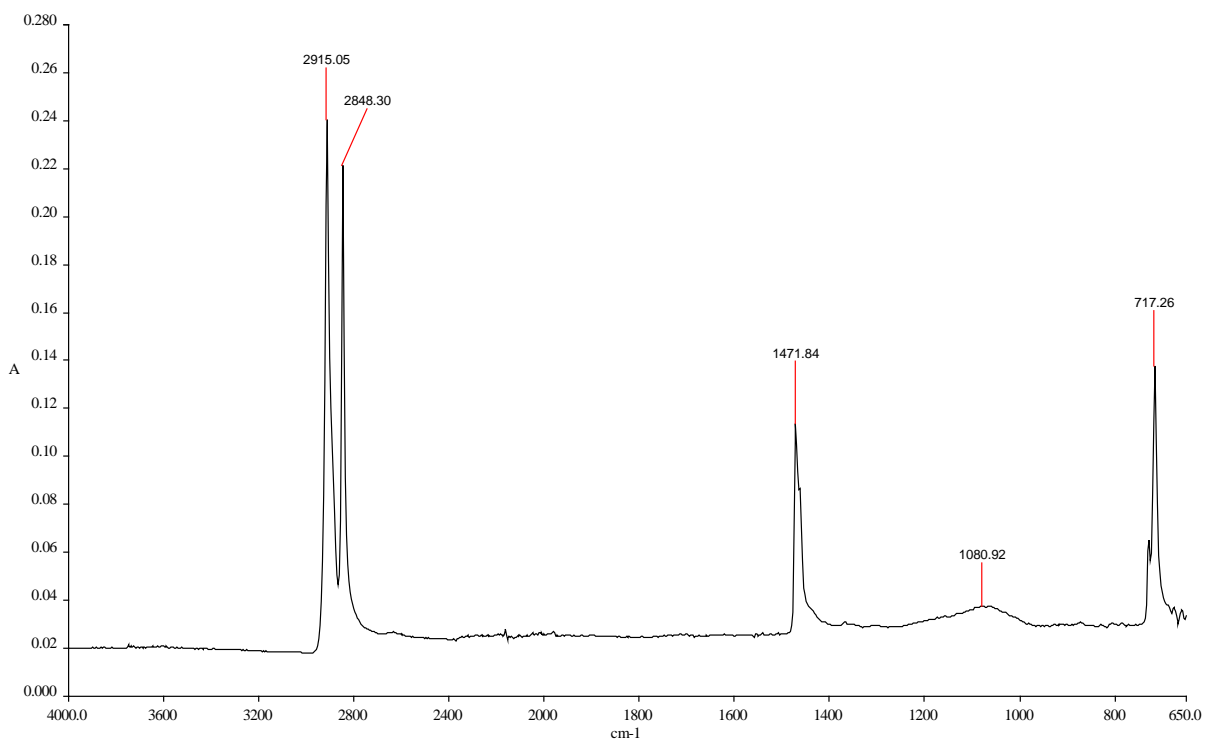
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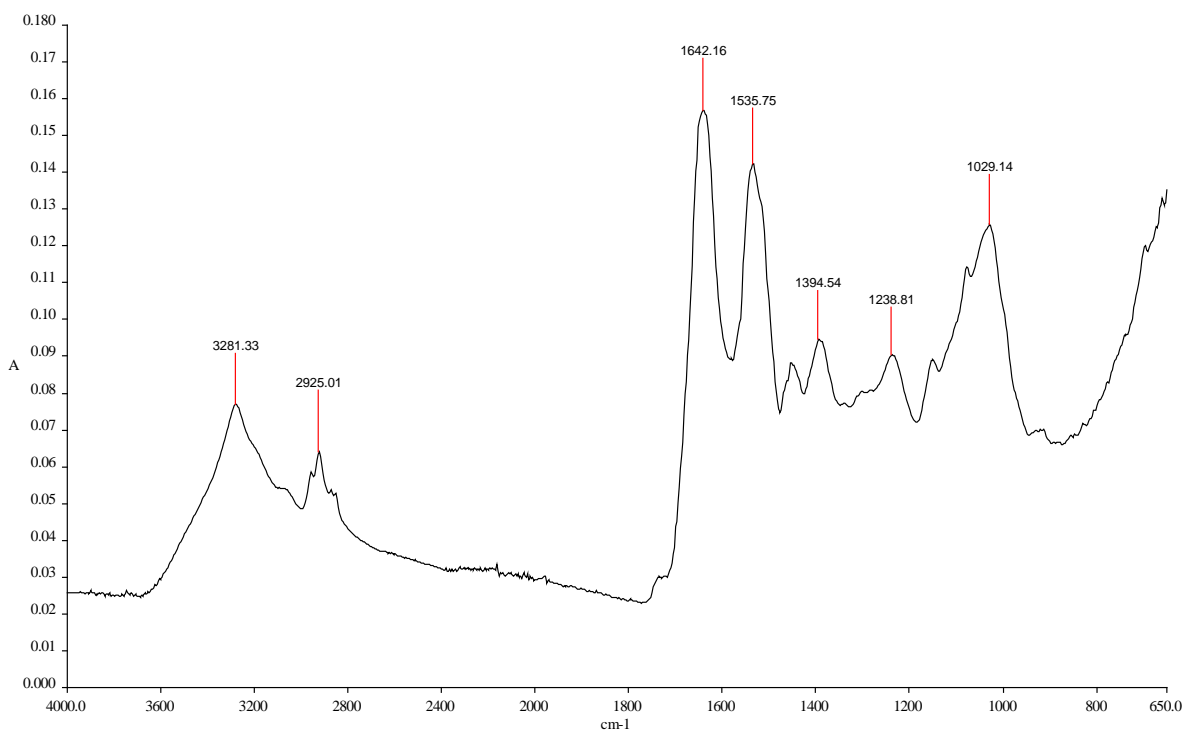
APPENDICES

FT-IR of PE, Spirulina, EG, PE-g-MA

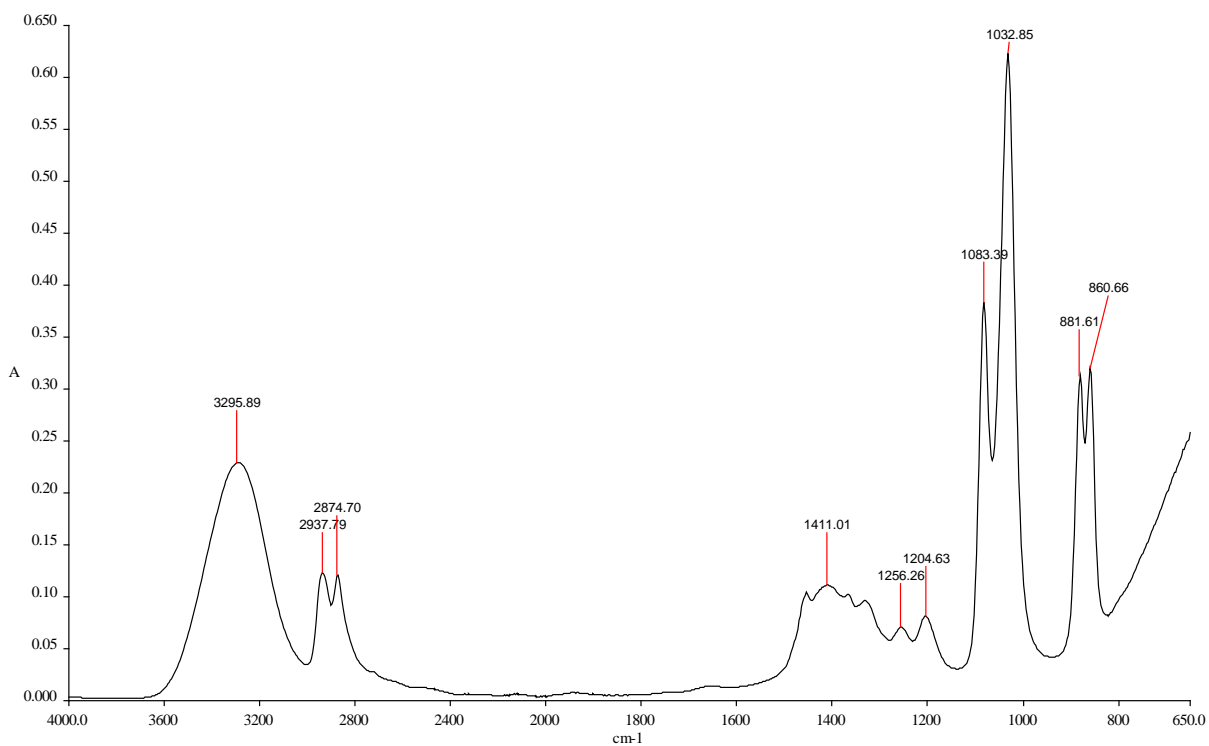
FT-IR of PE



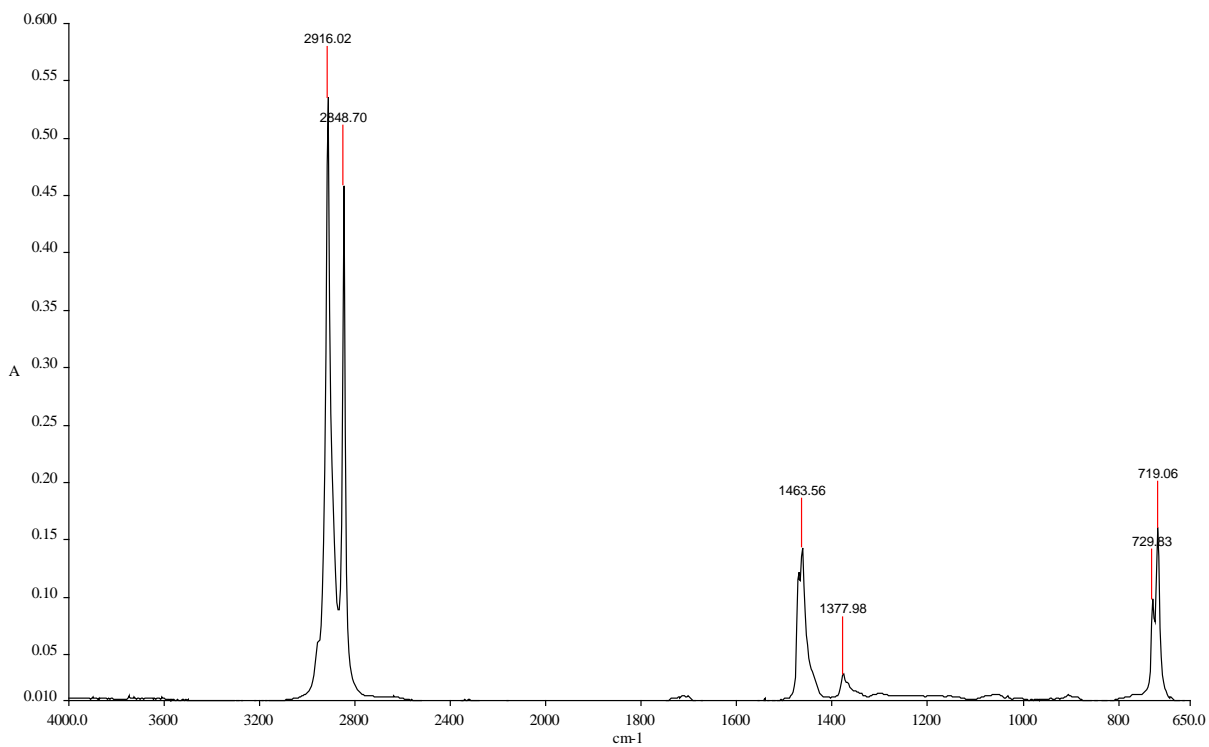
FT-IR of Spirulina



FT-IR of EG



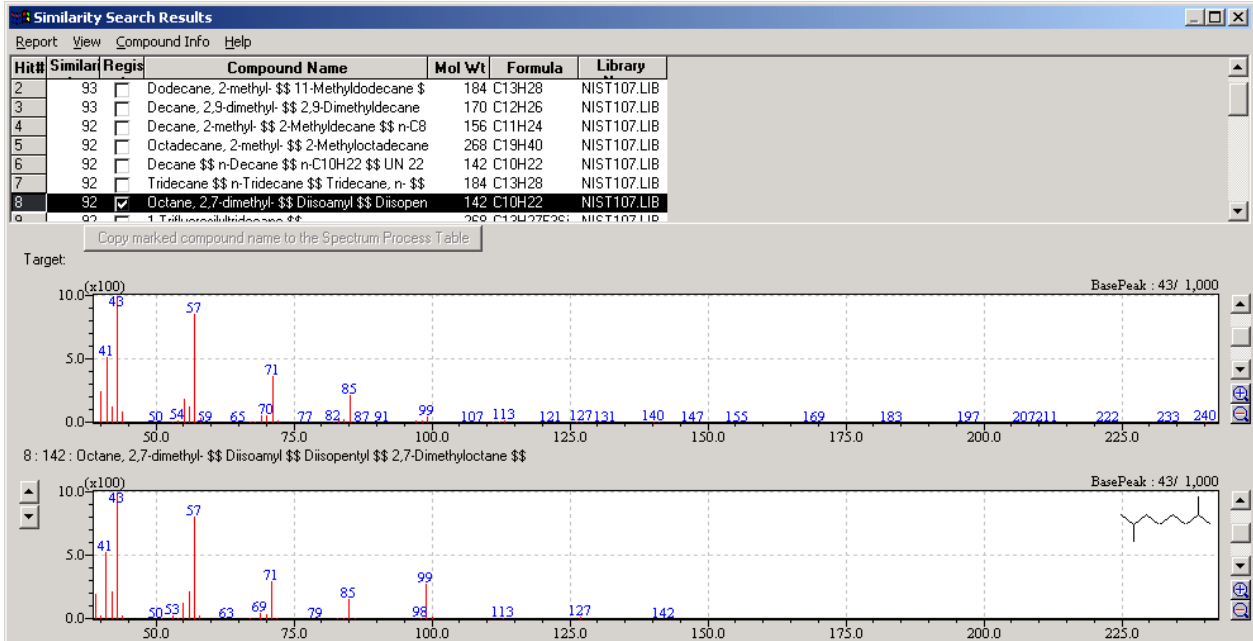
FT-IR of PE-g-MA



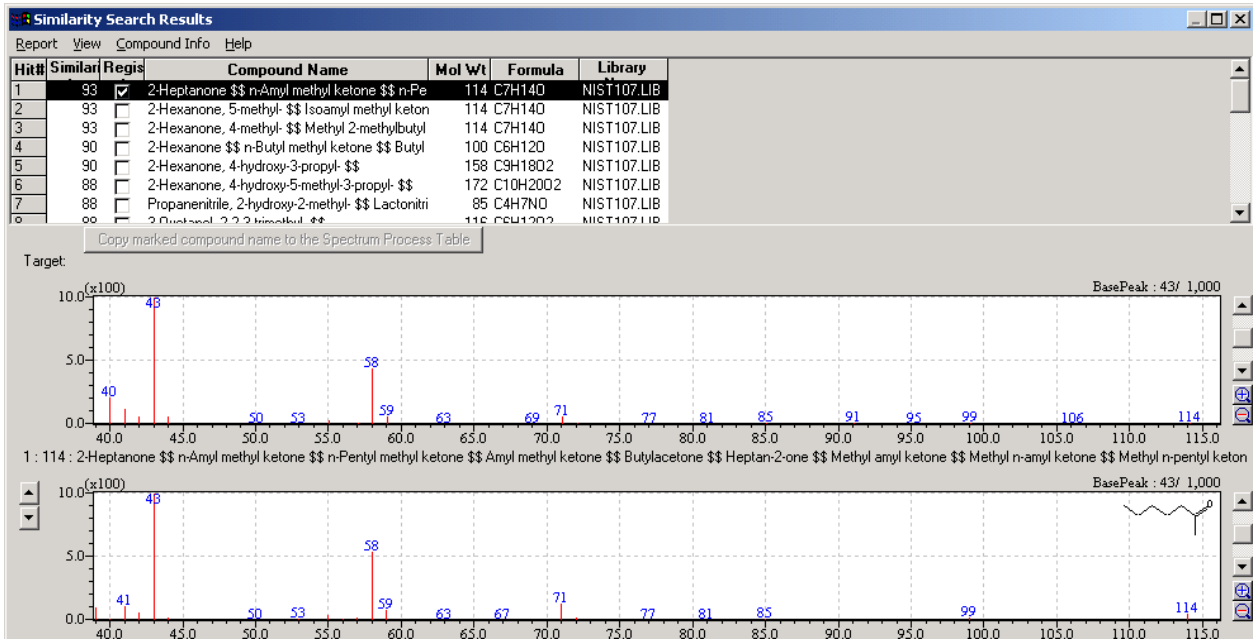
MS Spectra of Most Possible Compounds in Spirulina Bio-lastic

100 Spirulina (SP) Bio-plastic

At T=24.66, the compound has 92% possibility to be octane.

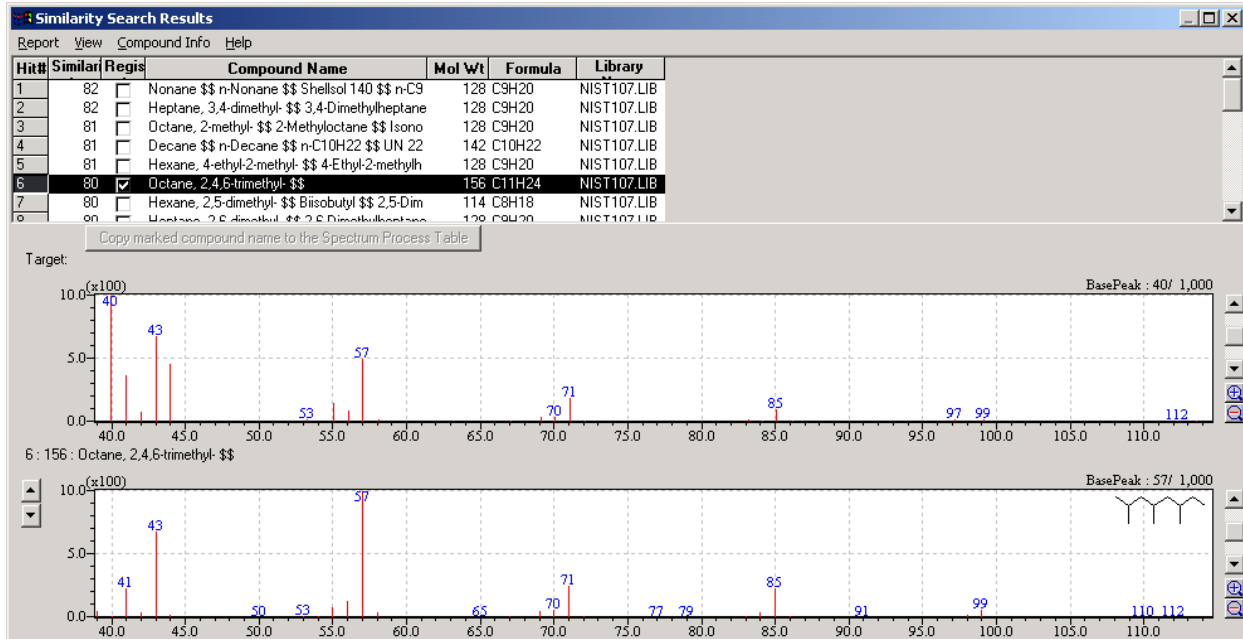


At T=8.94, the compound has 93% possibility to be 2-Heptanone.



95-5 SP-C Bio-plastic

At T=24.59, the compound has 80% possibility to be Octane.



At T=8.18, the compound has 80% possibility to be 1, 2- propadiene.

