IMPACT OF POLY (ETHYLENE GLYCOL) (PEG) CONCENTRATIONS IN POLY STYRENE BLENDS AS ANTI-ALGAL SUBSTANCE AGAINST GREEN MICRO-ALGAE

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DEDICATION

DEDICATED TO A ROLE MODEL AND MOST INFLUENTIAL FIGURE OF MY LIFE, MY GRANDFATHER MASTER MUHAMMAD NASEER QURESHI (LATE) WHO HAS THE BIGGEST SHARE IN MY TODAY'S SUCCESS. WHATEVER I AM AT PRESENT IS BECAUSE OF HIM & BELONGS TO HIM. MAY HIS SOUL REST IN HEAVEN FOR EVER. EQUAL SHARE FOR MY FAMILY, PARENTS, TEACHERS & MATCHLESS FRIENDS FOR THEIR ENDLESS CARE, SUPPORT & ENCOURAGEMENT.

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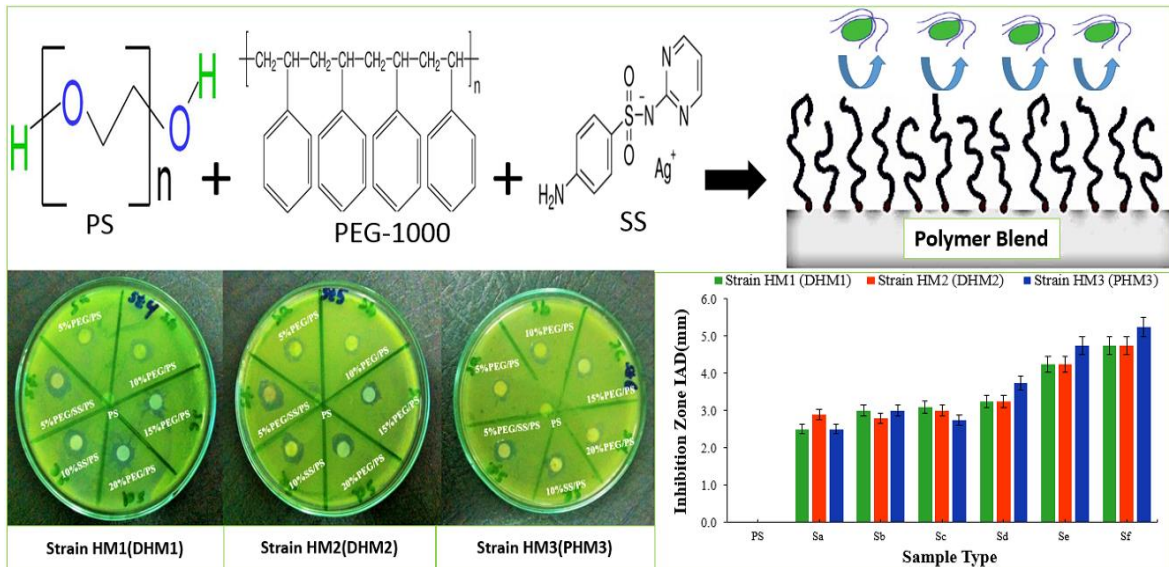
ABSTRACT

Algal Bio-fouling is one of the most important global issues that negatively effects diverse fields mainly food preservation, water purification, marine industry, heat exchangers, photo bioreactors, biomedical implants etc. There is a wide variety of different techniques and material systems employed to either inhibit the microbial growth or disinfect the underline biofilm. Among various anti-fouling materials polymers have gain special interest due to their economical availability, processing ease and efficient growth inhibition of microbial films. They can be used in a wide variety of forms including copolymers, blends, coatings, polymer brushes, grafted polymers etc. Poly (ethylene glycol) (PEG) is one of the efficient anti-fouling materials. In present study Poly (ethylene glycol) (PEG-1000) and Silver sulfadiazine (SS) are blended as anti-algal agents with Polystyrene (PS) in different weight concentrations. The weight concentration of PEG was varied from 5% to 20% in binary PEG and PS based blends. The specific blend of PS and SS was composed of 10% SS. There was also one ternary blend composed of PS, PEG and SS in which PEG and SS were used 10% each. The synthesis of various polymer blends was done through Extrusion Plastometer at 210C° and using 10kg load. The anti-algal behavior of polymer blends was studied through agar disk diffusion method against three different algal strains namely Dictyosphaerium sp. Strain HM1 (DHM1), Dictyosphaerium sp. Strain HM2 (DHM2) and Pectinodesmus sp. Strain HM3 (PHM3). The algal growth inhibition factors with respect to diameter IAD (a) and area IAD (b) were then calculated and plotted corresponding to various samples. The IAD (a) factor increased from zero to maximum value of 8.3mm for ternary blend of PEG, SS and PS. The IAD (b) parameter managed to increase from zero to 343.2mm^2 for the same sample. The results indicate that both PEG and SS based blends are effective against all the three algal strains. The synergetic effect of PEG and SS was exceptional as far as anti-algal properties are concerned. The algal growth inhibition was found to increase with the increase in amount of anti-algal agent. The samples were then characterized through other techniques like mechanical testing, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and zeta-potential (ZP). Mechanical testing was done to study the tensile properties like tensile strength, percentage elongation and elastic modulus of various samples. DSC and TGA were employed to analyze the thermal transitions and thermal stability of blends

respectively. The purpose of zeta-potential (ZP) measurement was to calculate the zeta potential and particle size of algal strains which are extremely significant parameters to design certain anti-fouling system. The results proved the anti-algal behavior of PEG and SS based Polystyrene blends with excellent thermal and mechanical profile which can be used for a wide variety of applications.

Graphical Abstract

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List of Abbreviations

PEG	Poly (ethylene glycol)
PS	Poly styrene
SS	Silver sulfadiazine
DMSO	Dimethyl sulfoxide
BBM	Bold Basal Medium
IAD	Inhibition of Algal Growth through Disk Diffusion
TGA	Thermo gravimetric analysis
DSC	Differential Scanning Calorimetry
ZP	Zeta Potential
Mob	Mobility
Cond	Conductivity
PD1	Poly Dispersity Index

INTRODUCTION

1.1 Back ground

Bio-fouling refers to the undesirable accumulation of a biotic deposit on a wetted surface. This biotic deposit contains various micro plants and animals namely micro-algae, bacterial, fungus and other such microorganisms. The macro organisms like macro-algae, barnacles, mussels etc. may also play their role in biotic film deposition along with typical microbial organisms in certain cases. The typical biotic deposition is a five stage process. Some of these stages may occur in parallel or not been followed by certain microbial organism at all [1]. The microbial film deposition is basically a surface phenomenon which effects diverse fields. The fields most effected by bio-fouling include food preservation, medical, water purification, heat exchangers, photo-bioreactors, marine and a number of other industries. As far as food preservation is concerned bio-fouling effects the quality and nutrient contents of food making it non-hygienic. The fouling related issues of water membranes reduce their flux and disturb the purity of water. The marine industry is the most effected field with respect to microbial activity. The reason being ideal conditions for bio-fouling to occur including suitable temperature, excess of water, sunlight and presence of large number of biotic species. Ship hulls, ship engine, marine platform etc. are severely affected by bio corrosion leading to higher fuel expenditure, extra drag and increased amount of stress, load & fatigue. Medical field is the most sophisticated field as far as fouling is concerned. These bio organisms can adversely affect various bio Implant (Dental, Orthopedic implant etc.), respirator, contact lens, catheter, biosensor etc. Biological corrosion also effects a number of other industries including Heat exchanger, Fluid flow (Pipes), Diesel Fuel, Food, paper and paint, Metal-cutting fluid, photo bioreactors etc.

If we specifically talk about micro algae it also inauspiciously effects various fields. The effects of microalgae are same as other microorganisms as far as bio corrosion and its damage is concerned. Infect in many cases a number of microorganisms like algae,

bacteria, fungus, debris, sediments etc. together play their parts in biofilm formation especially in marine industry. So microalgae effects the same fields that are disturbed by Bio-fouling in general. Some of the specific ones are:

- Food Preservation

- Marine Industry

- Water Membranes

- Bio Medical & Bio Implants

- Bio induced Corrosion

- Photo Bioreactors etc.

To combat Bio-fouling various techniques are used worldwide which include synthesis of biocides, non-toxic coatings, thin films and new bio resistant materials. These methods inhibit the growth of microbial organisms. There are some methods which are used for cleaning or disinfecting purposes once the bio film is developed. They actually don't inhibit the growth but rather remove the bio film once it is developed. Some of these methods include usage of Biosensor, Filters Electric Current, Surface charge, Responsive surface, Mechanical Jet Spray, Explosives, Vibrations, Plasma , rays etc. to clean away bio film through various mechanical, physical ,electrical and chemical methods.

1.2 Scope of Study

The adverse effects of algae are already discussed. With the main focus on micro-algae the idea was to develop functional anti-fouling system. It is an established fact from the research literature that the anti-fouling system effective against certain type of micro-organism like algae may also be effective against other species like fungus or bacteria because a number of fouling organisms share the same properties especially the surface characteristics. For example majority of biological species are hydrophobic. Development of some hydrophilic system would be equally effective against all those species which are hydrophobic. In Pakistan there are various issues related to food preservation, water

purification, bio medical, corrosion, energy, marine Bio-fouling etc. There is already shortage of food and a large number of people cannot afford proper meal. In such scenario there is no reason to spoil or waste food. Eating unhygienic food may also be problematic. A large section of society is not facilitated with clean and pure water, though there are huge water reserves. The need of hour is to purify the water from reserves. Water membranes could be one of the systems to purify water. But there are fouling related issues involved in which also includes micro-algae. Similarly there are corrosion and marine Bio-fouling related issues. Micro-algae is definitely involved in marine bio corrosion which causes extra fuel consumption, and material loss due to extensive fatigue and drag. This also leads to the loss of money making the processes highly expensive.

The photo bioreactors are among those industries which positively use bio mass to convert it in to biofuel which can be used to produce energy and electricity to be used for useful purposes. For example algal bio reactors use micro algae as bio mass which is a best option against other microbial and biological species as far as efficiency and quality is concerned. But these reactors are also prone to Bio-fouling in which algal bio mass sticks to the reactor tubes as the process continues. This does not allow light to further come in which is necessary for algal growth. The plant ultimately stops processing bio diesel. These are only a few areas to name. There is a large list of the systems badly effected through Bio-fouling or microbial corrosion. The idea adopted by the author is to develop functional anti-fouling polymers compositions for micro-organisms with the main focus on micro-algae. There is definitely need to develop new anti-fouling systems that are economical, nontoxic and efficient.

1.3 The Present Approach

The author focused on developments of functional anti-fouling polymer compositions to combat algal bio fouling.

Motivation: Polymers are among the best and most widely used materials to inhibit microbial growth. A wide variety of polymers used as anti-fouling or anti-algal systems are found in literature. Some of the typical examples include Poly Ethylene glycol(PEG),

Poly ethylene oxide(PEO), Poly Vinyl Acetate(PVA), Poly acryl amide(PAA),Poly methyl acrylate(PMA),Poly methyl meth acrylate (PMMA), Poly urethane (PU),Poly glycerol (PG) etc. They can be used alone or in combination with other materials in various forms which include:

- Blends
- Co-Polymers
- Coatings
- Thin Films
- Grafting
- Polymer Brushes
- Polyelectrolytes etc.

In present research work Poly (ethylene glycol)(PEG-1000) and Silver sulfadiazine (SS) based blends of Polystyrene (PS) were used as anti-algal systems. A total of six blends were synthesized. PEG-1000 was used as main anti-algal agent in four of the blends. The weight % of PEG varied from 5 to 20% in these blends while remaining two blends consisted of SS. One of the blends consisted of 10% SS by weight along with PS. The last one was ternary consisted of 10% SS and 10% PEG with remaining amount of PS. Their anti-algal properties were then studied through Agar disk diffusion method followed by other characterization techniques like DSC, TGA, Mechanical Testing, UV-Visible Spectroscopy, Optical Microscopy, Zeta potential, and Dilatometry.

The surface properties of certain anti-fouling system are extremely important in generating anti-fouling properties. Fouling organisms are generally hydrophobic, so are micro-algae with higher values of contact angle. Thus the surface prone to bio-film deposition should be hydrophilic with lower values of contact angle and surface energy.

The anti-algal agent used in the present case PEG-1000 is also hydrophilic with polar functional groups. It has reasonably lower values of surface energy and contact angle. The system is also nontoxic, economical and efficient, because the blend components are commercially available at economical prices. There is a variety of processing methods to synthesize polymer blends that are simple and economical too. Agar disk diffusion method proved the excellent anti-algal properties of polymer blends. The blends have tendency to

be used in a variety of fields such as Food Preservation, water purification, photo Bioreactors etc.

Chapter 2

LITERATURE REVIEW

2.1 Fouling

Fouling is a broad term indicating a number of different material degradation processes including bio fouling, particulate fouling, in-organic fouling, organic fouling, crystallization, chemical reaction fouling, corrosion, solidification etc. [2]. The major types include organic-fouling, inorganic-fouling, particulate-fouling and the most important one bio fouling.

- **Organic-Fouling**

  Organic-fouling is actually the deposition of unwanted organic materials like proteins, fats, oils etc. on certain material surface.

- **Inorganic-Fouling**

  Inorganic fouling is associated with the accumulation of inorganic materials like scaling. It can also be linked with general metallic or polymer based corrosion involving oxides of in-organic materials.

- **Particle-Fouling**

  Particle-fouling involves the accumulation of various particles including silt, clay, dust, sand etc. on certain material surface.

2.2 Bio-fouling

Bio-fouling is the undesirable accumulation of biotic deposit on the surface of certain material. This biotic deposit may be either plant or animal based ranging from various microbial organisms like microscopic bacteria, cyanobacteria, algal spores, barnacle cyprids, fungi, unicellular eukaryotes etc. to larger marine invertebrates and macro alga etc. These bio-organisms mostly include water borne and aquatic species. Such accumulation leads to bio- corrosion of the various structures thus effecting their output and performance.

Bio-fouling thus may be either microbial based or combination of micro and macro bio-fouling caused by synergetic effect of microbial and macro-organisms respectively. Algal Bio-fouling is a specific type of bio degradation initiated by micro algae in highly humid and aquatic environments. The algal cells not only multiply in their number but also lead to the attachment of other biological species forming a complex heterogeneous system of microorganisms which may lead to macro bio-fouling [3]. Bio-deterioration adversely effects food processing, water membranes, medical implants and a number of other important industries which include marine industry, paper manufacturing, underwater construction, and desalination plants, photo bioreactors. etc. [4].

2.3 Factors Effecting Bio-fouling

The biological degradation of certain material depends specifically on its physical and chemical properties, the type of micro-organisms and environmental conditions. The environmental factors i.e. aqueous and high humidity conditions support the deposition of these unwanted microbial films causing physical, chemical, and biological degradation to material surfaces [5–10]. Bio-fouling and its severity is dependent on type of microbial organism, thickness of fouling layer and most importantly local environment. All of these variables are different for medical, marine and industrial fields. The fouling associated with medical field is mostly bio-fouling. The fouling in industrial and marine fields may include organic, inorganic and macro fouling [11–14]

2.4 Morphological Characterization of Bio-films

The morphological properties of biofilms are characterized by the density, composition, thickness, structure, adhesive strength and weight of fouling organisms through various sophisticated equipment like transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force and fluorescence microscopy techniques etc. This can indirectly measure us the amount and severity of bio-fouling [15–20].

2.5 Mechanism of Bio-fouling

The growth of microbial organisms on certain surface is a complex phenomenon which is not completely understood yet. Marine industry is the most adversely effected field. Marine Bio-fouling generally occurs in following five stages.

- The first stage involves adhesion of microbial organisms forming primary film on the surface.
- In the next stage the transport of microbial cells and the immobilization of fouling organisms occur on to the surface.
- The third stage is linked with the microbial attachment to the substratum and consolidation through extracellular polymer production. This actually produces main fouling layer on the surface.
- In the next stage the development of complex community of microalgae, bacteria, debris, sediments etc. occur on the surface in the presence of multicellular species, on the surface.
- Until now in previous stages micro fouling has occurred. The last stage corresponds to macro fouling which involves the attachment of macro marine organisms like barnacles, mussels and macro-algae etc. It is not necessary that all of the above mentioned stages occur in certain. Some of these stages may occur in parallel or not been followed at all by certain microbial organisms [21].

2.6 Prevention Techniques against Bio-fouling

Bio-fouling causes heavy economical losses. These loses are as much as 5-10% of production costs which is a serious issue to think about. Various methods are used to prevent or reduce microbial activity which include development of various anti-fouling systems and materials. Some of the prevention techniques are described below.

2.6.1   Biocides

Biocides are specific chemical substances that can inhibit the growth of microorganisms responsible for microbial activity. The incorporation of biocides into an anti-microbial

coating is done either through physical or chemical modification of the underline surface. The tributyltin also abbreviated as (TBT) is one of the most commonly used biocide [22].

2.6.2   Nontoxic Antifouling Coatings

Another most commonly used prevention method against bio-fouling is to use non-toxic coatings that should be anti-sticking. These coatings being good alternative prevent the negative effects of biocides. The coatings are mostly organic or polymer based [23]. Some of the materials used as coatings to inhibit bio-fouling include Hydroxyapatite, PEG, antibodies, oligo ethylene glycol, zwitterionic polymers, fluoropolymer and fluorosilane, self-polishing copolymer, hydrogels etc.

2.6.3   Anti-Fouling Polymers

It is important to mention polymers are among the best and most widely used antifouling materials. Only few polymers used as coating are named here but there is actually a large number of others being used as antifouling agents. Polymers are also used in the form of blends, copolymers, grafting, polymer brushes, thin films etc. to give exceptional anti-microbial properties. Among all materials polymers are most versatile and significant anti-fouling systems. The details of anti-fouling polymer systems are discussed in details in later sections of literature.

2.6.4   Energy Methods

These methods use energy to kill microorganisms. They have tendency to finish out the organisms in short span of time. Some of these methods include.

- ✓ Pulsed Laser Irradiation
- ✓ Plasma Pulse Technology
- ✓ Ultrasonic Transducers [24]
- ✓ High Energy Acoustic Pulses down Pipes [25]

The major disadvantage of these methods is that these systems are not effective against wooden-hulled boats, or boats composed of a soft composite material. These do not inhibit the microbial growth but rather clean away the film once it is deposited.

2.6.5   Other Methods

Besides these main methods there is a wide variety of other methods that are used to inhibit or clean the microbial growth. Some of these are:

(a) **Biosensor:** Bio sensors can be used for the detection of bacteria

(b) **Filters:** Filters basically cause disposal of microbial organisms

(c) **Electric Current:** Electric current or field has tendency to inhibit microbial growth.

(d) **Surface Charge:** Surface charges play important role in repelling the opposite charged fouling organisms.

(e) **Responsive surface:** Bio-fouling is basically a surface phenomenon. If we somehow manage to play with the wettability of surface using the parameters like light, temperature, solvent, electric field, etc. formation of bio film can be inhibited.

(f) **Mechanical:** Mechanical methods involve various mechanical techniques to remove biofilm.

(g) **Jet Spray:** In this method removal of microbial film is done through pressurized water blasting.

(h) E**xplosives:** The cleaning of bio film can be done through explosion.

(i) **Vibrations:** Ultrasonic vibrations have tendency to disturb the microbial growth.

(j) **Plasma:** Cool plasma can also be used to clean the biologically damaged surface.

(k) **Gamma rays:** An exposure to gamma rays can washout the unnecessary biofilm deposition.

2.7 Impact of Bio-fouling

Microbial growth adversely effects various fields including food preservation, water purification, photo-bio reactors, marine industry, bio implants, bio sensors, paper production, cooling towers, heat exchangers etc. Studies have identified more than 4,000 different species of marine organisms only.

There is not only difference in their adhesion mechanism but also their adhesive composition. Therefore combating biofouling is a considerable technical challenge globally. [26- 28]. Some of the most important fields and their specific harmful impacts are listed in Table 1.

The direct or indirect economic losses related to marine industry are huge with estimated equivalent to 7% of the gross national product [29]. Marine bio fouling directly effects ship hulls, marine platforms, jetties, offshore rigs etc. [30-33]. It leads to increased fuel consumption, dry-docking cleaning expenses, loss of hull strength, bio corrosion, etc. As an example, the cleaning operations in aquaculture framings as a result of bio fouling leads to the estimated increase of 20% in fish production cost [34]. The bio fouling is also one of the major problems in heat exchangers which effects their heat transfer efficiency, leading to higher operational and maintenance costs. [35].

Membrane bio reactors are very innovative tools used for waste water treatment. [36–38] but they are also prone to microbial attack which directly affect their membrane life, flux, water quality and operational costs [39–41]. As polymers find wide variety of applications in food packaging, they can easily be attacked by various fouling organisms prone to severe transmitted diseases. Microbial growth can severely affect efficiency, productivity, and food quality [42].

Bio-fouling leads to the performance impairment and functional loss of biomedical devices. For example biotic film causes nonspecific protein adsorption and high nonspecific signal in biosensors [43, 44] which affect their sensitivity, selectivity and life time leading to the sensor failure [45].

Table 1: Specific Harmful effects of Bio-fouling in various fields & Industries

Industry	Specific Harmful Effect
Medical	
Orthopedic implant	Infection causing its removal at the end
Respirator	Ventilator related issues
Contact lens	Eyes Infection
Catheter	Urinary tract infections
Hemodialysis	infectious break-outs
Dental implant	Periodontal disease, Dental infections
Biosensor	In accuracy of Results
Marine	
Ship hull	Higher fuel expenditure
Ship engine	Extra drag creating increased stress
Marine platform	Extra amount of stress, load & fatigue
Industrial	
Metal	Metallic bio-corrosion
Water membrane	Decreased flux recovery
Heat exchanger	Lower efficiency
Fluid flow(Pipes)	Friction leading to pipe losses
Drinking water	Poor quality of water
Diesel Fuel	Quality of fuel
Food, paper and paint	Poor quality food quality & health related issues
Metal-cutting fluid	Filter blockage & health risks

2.8 Bio-fouling Organisms

In marine environments, more than 4000 microbial organisms are associated with fouling problems. There is not only difference in their adhesion mechanism but also their adhesive composition. Bio-fouling organisms can be broadly classified in two types namely macro and micro bio-fouling organisms which includes various species of algae, bacteria, fungus, barnacle cyprids, unicellular eukaryotes, larger marine invertebrates, macro alga etc.

2.9 Algae

There is no basic single definition of algae. Algae have chlorophyll which is their basic photosynthetic pigment and lack a sterile covering of cells around their reproductive cells. Algae are a diverse class of plantlike microorganisms which employ photosynthesis to produce food. The difference between algae and plants is related to their cells which are not clearly organized into different types of tissues having various functions. Algae trap sunlight due to chlorophyll and other pigments contained in it. The trapped light then undergoes photosynthesis in which stored energy is converted into food molecules e.g. carbohydrates. Algae may be unicellular or multi cellular. The unicellular organisms include Chlorella , diatoms etc. as against multicellular types like giant kelp etc. The multicellular type of algae don't have stems, leaves, or roots just like complex and higher plants. The algal cell walls are made up of cellulose and pectin, due to which they have slimy feel [47].

Algae are polyphyletic which means that these microorganisms may or may not be always closely related to one another. All members of the certain algal group may not have the same common ancestor. They can grow in fresh or saltwater. They also occur on moisty surfaces of soil or rocks. Micro-organisms may be either eukaryotes or prokaryotes. As against other similar microorganisms like bacteria and fungus, algae are eukaryotes because DNA in their cells is contained within their nucleus enclosed by a membrane. Prokaryotes are opposite to eukaryotes whose cells do not have a nucleus which mainly include bacteria [47].

2.10 Classification of Algae

Broadly Algae can be classified in to seven different categories as described below. The classification is based upon the types of pigments used for their photosynthesis, cell walls characteristics, carbohydrate compounds stored for their energy, types of flagella used for their movement etc.

▪ **Phytoplankton:** It is a type of microscopic algae which reside suspended in water.

- **Zooplankton:** Zooplankton are tiny animals which drift through the upper surface of water bodies. They feed on phytoplankton.

- **Euglenoids:** The Euglenoids is a class of algae mostly occurring in fresh water. They are single celled, protozoan like species with no cell wall. Most of them are capable of producing their own food employing solar energy but they can also survive in dark if fed with organic matter. The others are heterotrophic which cannot produce their own food, instead rely on organic material suspended in the water.

- **Golden-brown algae:** Golden-brown algae and diatoms are single-celled algae that contain yellow pigments. They live in both fresh and salt water. Their cell walls are mostly composed of pectin filled with silica and don't contain any cellulose. The cell walls are quite rigid due to silica filled pectin. They store energy in the form of carbohydrate and large oil droplets. Diatoms poses glass shells which are made up of silica. These organisms are roughly 40,000 to 100,000 in number.

- **Fire algae:** Fire algae belongs to a class of single-celled algae including dinoflagellates, which have two flagella used for locomotion. A wide variety of these algal organisms live in salt water, with others residing in freshwater. When exposed to air some of these organisms emit bright light, interestingly which at night look like fire on the ocean's surface. Therefore, these species are called fire algae.

- **Green algae:** Green algae, also known as Chlorophyta, are found in fresh or sea water. Some of these species are single celled microscopic entities only able to be seen under microscope. They form slimy green scum in stagnant ponds. Remaining are much complex and larger. They are composed of spherical colonies consisting of many cells. They may also contain straight or branch patterned filaments with long and thin series of cells. Green species are also believed to give birth to first land plants.

- **Red algae:** The red algae also called Rhodophyta are marine plants that live mainly in shallow waters and deep tropical seas. A few also occur in freshwater. Their body forms range from single-celled to branched filaments. The larger species have filaments that are massed together and resemble the leaves and stems of plants. They have no flagella and typically grow attached to a hard surface or on other algae. Some species contain a red pigment; others range in color from green to red, purple, and greenish-black.

- **Brown algae:** The brown algae also known as Phaeophyta, are shiny brown seaweeds that are especially abundant along rocky coasts, although some float in the open ocean. Brown algae are large in size and include the giant kelps, which are located along the Pacific coast and form forests that provide habitat to a wide range of marine life. Brown algae contain an accessory brown-colored pigment that gives the plants their characteristic dark color.

- **Yellow-green algae:** The yellow-green algae, or Xanthophyta, primarily occur in freshwater. They can be either single celled or form colonies. Their cell walls are made of cellulose and pectin compounds that sometime contain silica. They can have two or more flagella for locomotion and store their energy as carbohydrates. Their yellow-green color is derived from from the pigments carotenoids and xanthophyll [48].

2.11 Number of Algal Species

The exact estimate about number and distribution of various algal species cannot be made accurately because there are thousands of algae types found around different parts of world. For example US National Herbarium takes on approximately 320,500 different dried specimens of algae among which 669 different types of marine species were found just in California [49]. This large number can give us a rough estimate about the number of algal species researched in America, only in one part of world [50]. The estimates about different types of species vary widely in different parts of world. For example, UK Biodiversity Steering Group reported the presence of almost 20000 algal species in United Kingdom [51] . Estimates tell us that there are about 5000-5500 species of red algae across the world. There are about 1300 different algal species in Australian waters [52]. Different studies reveal the presence of 400 seaweed species in western coastline of South Africa, [53] and almost 212 different types in KwaZulu-Natal [54].

There is a wide discussion about the correctness of these results. These surveys keep on going all the time and there is a large variation in results of these studies. For example, the most recent study estimates the presence of approximately 72,500 algal species worldwide [55].

2.12 Distribution of Algae

Algae spread mostly through the dispersal of spores. In other words spreading of algae is associated with the dispersion of Plantae by seeds and spores. Spores are found everywhere across the earth, free-floating and precipitated with dust like atmosphere, fresh and marine waters, humans etc. The growth of a spore into an organism depends on some factors like, the environmental conditions of the place where the spore lands and the combination of the species. The spores associated with fresh water algal species are scattered mostly by living carriers, wind, running water etc. [56]. The waters taking these spores are actually chemically selective. Currents cause the marine spores to disperse.

The distribution of algae across some parts of world is also possible due to some floristic discontinuities but on smaller scale. Floristic discontinuities are actually caused by various geographical characteristics e.g. Long distances of oceans or general land masses, Antarctica etc. It is therefore feasible to identify algal species according to their location e.g. "Pacific Algae" or "Red Sea Algae.

2.13 Nutrient Content

The nutritional value of algal specie depends on its specific type. Dried algae is generally nutrient rich having 5 percent water in comparison with raw one containing more than 80 percent water. Most of the Algae types are rich in various nutrients like copper, iron, thiamine, riboflavin, manganese, vitamin, protein, beta carotene, iron etc. [57].

2.14 Potential Benefits & Side Effects

2.14.1 Benefits of Algae

- Agar is a specific growth medium obtained from red algae. It has a variety of commercial applications [58]. For example agar is an excellent culture medium for the laboratory growth of bacteria and fungi because most of the microorganisms are not capable of digesting agar. Agar is also used in various desserts and soups and in the manufacturing of cosmetics & pharmaceutical.

- Alginate also known as Alginic acid is a commercially important material derived from brown algae. It is used as a gelling agents in food and medical dressings. It is used as stabilizer and thickening agents in the food and pharmaceutical industries.

- If bio fuels are taken serious for resolving energy crisis across the globe, they can equal or even strike the cost level of fossil fuels. If we talk about algae based fuels specifically, they have capability to produce more biomass per unit area than any other form of biomass in a year.

- Various types of algal strains are used as fertilizers, soil conditioners & livestock feed [59]. Microscopic & aquatic algal species are cultured in ponds & clear tanks and are either harvested or used to treat effluents pumped through the ponds.

- Algae is a national food of many countries. Various types of algae are also used in foods. For example Natural seaweeds, a specific algal specie is an important food source in many parts of Asia which is an important source of potassium, iodine, magnesium, iron, calcium, vitamins etc. [60]. More over some commercially cultivated microalgae and cyanobacteria species, are used as nutritional supplements. Laver is used to make a specific type of laver bread.

- Scotland, Ireland, Greenland and Iceland use Sea lettuce and badder locks as an important salad ingredient. Algae is an important source of omega-3 fatty acids. China uses around 70 algal species as food, Fat choy, is a type of cyanobacterium which is considered as a vegetable there. Japan uses about 20 species as food [61]. Various algal strains are also used as food in other parts of world e.g. North America, California, British Columbia, Hawaii, New Zealand etc.

- Algae has great potential to reduce the application of large amounts of toxic chemicals in sewage that would be needed otherwise. Algae can capture fertilizers in runoff from farms. The enriched algae can itself be used as a fertilizer during subsequent harvesting. Filtration of aquariums & ponds can be done using algae [62].

- Agricultural Scientists researched that 60–90% of nitrogen runoff and 70–100% of phosphorus runoff can be captured using a horizontal algae scrubber or ATS. They also found that algae can be used to reduce the nutrient drainage from

agricultural fields that has potential to increase the quality of flowing water in oceans, rivers and streams.

- Algal species are gifted with natural pigments like carotenoids & chlorophylls. These natural pigments can be used as an alternative to artificial coloring agents and chemical dyes [63].

- Different algal strains are used as stabilizing agents. For example Carrageenan obtained from Chondrus crispus (Type of Red alga), is used as a stabilizer in various milk products.

2.14.2 Potential Side Effects of Algae

- The harmful algal bloom in water can cause serious problems like bad taste and odour, formation of disinfected by-products and clogging of filter beds [65].

- The cyanobacteria blooms, especially microcystis aeruginosa are negatively effecting aquatic environment due to presence of very potent toxins [66] that cause high turbidity, anoxia, fish kills, and food web alterations [67]. Algae and fungi based colonization causes loss in bulk properties.

- As far as the effect of algae on drinking water is concerned it adversely impacts the human and biological health. The algal bio mass sticks to the filter surface which not only shortens the filtration cycle but also penetrates in to the water supply pipe network which badly effects the quality of water [68].

- Algae can often bring side effects to our health. Most of the health related issues take place if algae is used as food. It can sometimes cause various allergic problems e.g. difficulty in breathing, swelling, rash and anaphylaxis.

- Sodium is not good enough for people suffering from high blood pressure or certain heart disease. Algal strains are high in sodium content which may be problematic at times if such people consume algae as food. Spirulina a specific algae food is not a healthy item for people suffering from autoimmune disease or phenylketonuria. It can suppress immune function and blood thinners.

- Contaminated algae products are dangerous to use. For example microcystin is a contaminant that can cause kidney, liver and brain problems. Contamination related to

cyanotoxin can result in, heart, seizures, pancreas and respiratory problems. Some contaminated products can also cause vomiting, thirst, nausea or stomach pain.

- It can also cause various skin reactions and gastrointestinal effects. High iodine, dried seaweeds can potentially increase the amount of thyroid-stimulating hormone in our body resulting in skin outbreak or yellow tint.
- Some algae types are themselves toxic, resulting in weakness, tingling, numbness, nausea, diarrhea, or even death [69].

2.15   Algal Bio-diesel

The demand of alternative energy sources and fuels has increased rapidly in the past few years. In the recent years, several substitutes have come into existence and many of them are sustainable and are replacing conventional fuels to some extent. Biofuel is one of those fuels which is rapidly becoming significant alternative to depleting fossil fuels. Bio diesel as an alternative substitute to traditional energy resources have many advantages. For example the combustion properties of biodiesel are very near to those of petro-diesel. Biofuels are generally nontoxic, highly biodegradable and renewable as well.

Microalgae is among the finest sources of bio diesel production. The basic idea is using sunlight to grow algae. Once algae is harvested, the next step is the extraction of fatty content of the cells and its conversion to diesel fuel. So sunlight first grows microalgae in specific conditions which is then used for bio fuel production. Bio scientists reveal that Micro algae has capability to produce 30 times more energy per unit area than first and second generation biofuel crops. US energy department reveals that microalgae can process 100 times more oil per unit acre in comparison with other similar biofuel crops [70].

2.16   Algal Biodiesel VS Crops

Microalgae can produce more fuel oil per hectare than other biodiesel crops like canola, soy and rapeseed. The reason is that in regular crops, all of the plant is wasted except seed which is the main ingredient. During plant growth energy, time and water are also wasted. This waste is very low in algal farming. The main lipid layer taking part in growth is actually a large part of the cell. This contains high percentage of the algal mass compared to a canola plant and its seeds [71].

2.17   Advantages & Drawbacks of Micro-algae as Bio fuel

2.17.1 Advantages of Algal Bio-fuel

Using microalgae as a bio diesel has numerous advantages like:

- High Efficiency & biomass Production
- Rapid growth of Algal blooms against crop plants even in over night
- High Photosynthetic
- Microalgae do not need any specific land and have capability of growing anywhere, even in brackish saline water.
- It can be harvested in tanks for example which can be placed anywhere, even in deserts having plenty of sunlight.
- Instead of fresh or drinking water, we can even use sea water for its production
- Once biodiesel extracted, the remaining matter can also be used as mulch, feedstock or fertilizer.
- There are minimum environment related issues using algal bio fuel because it absorbs large amounts of carbon dioxide for its production, thus reducing global warming and greenhouse effect. Studies found that one gallon of bio oil production from algae needs almost 14KG of carbon dioxide, so it does not have
negative impacts on environment [72-73].

2.17.2 Drawbacks of Algal Bio-fuel

- Being in research phase, Bio diesel production is expensive so far.

- There is not a wide variety of processing methods. The most common ones are open pond systems. In these methods, growth of algae sometimes become extremely difficult.

- Conventional open pond systems have problems related to evaporation, viral infection and low energy density oil production.

- Closed systems like photo bio reactors are alternative to open pound systems which produce high quality bio fuel. But the main problem is constant supply of carbon dioxide which makes these systems quite expensive.

- The most important disadvantage of photo bio reactors is the sticking of algae along the sides of reactor tubes which do not allow sun light to come in. So the production of bio diesel stops. The cleaning of tubes is difficult and time consuming because it needs plant shut down. So reactor runs for a short span of time and then automatically stops due to algae sticking alongside tubes. Process becomes low efficient and expensive.

- High risk of contamination due to pumping of Carbon dioxide into the ponds.

- Algal bio diesel is not practically tested in real time on large scale. The process is in research phase yet.

- No obvious information or research data about output, mileage and efficiency of algal fuel systems.

- Micro algae is prone to disease and can be attacked by different types of viruses. But the good thing is that infected algae can be still processed to fuel as against other crops which are completely destroyed by certain diseases before their fruit.

- Algae absorbs carbon dioxide during bio fuel production which ultimately is lost to the environment as the process ends, causing some pollution related issues. If carbon dioxide is blocked from going outside in to the environment, then micro algae can reduce greenhouse effect to great deal [74].

2.18   Algal Photo bioreactors

 A photo bioreactor consists of a closed system having a controlled environment used for the production of micro algae and then bio fuel. The basic principles behind photo bio reactors is using photosynthesis to generate biomass from light and carbon dioxide from algae. It provides higher growth rates and purity levels. Being a controlled system all of the parameters are optimized. Photo bioreactors have optimum temperature, carbon dioxide supply, light exposure, culture density, water supply, PH levels etc. [75]. The main advantages of photo bioreactor systems include lower water loss by evaporation, minimum contamination and maximum areal productions [76].

2.19   Advantages of Photo Bioreactors

- Being a controlled environment the production is high
- Photo bio reactors have high surface to volume ratio, so they are capable of using maximum amount of light thus providing maximum efficiency.
- Minimum Evaporation of water
- Constant Temperature
- Excellent protection against contamination
- Photo bio reactors can be mounted horizontally, vertically or at certain angle. Space saving is thus possible
- Minimum amount of bio Fouling

2.20   Disadvantages of Photo Bioreactors

- High capital cost
- High production cost
- The most important disadvantage of photo bio reactors is the sticking of algae along the sides of reactor tubes which do not allow sun light to come in. So production of bio diesel stops. The cleaning of tubes is difficult and time consuming because it needs plant shut down. So reactor runs for a short span of time and then automatically stops

due to algae sticking alongside tubes. Process becomes low efficient and expensive [77-78].

2.21   Antifouling Polymer Systems

Among various materials to combat bio fouling, polymer based anti-fouling systems are among the best anti-microbial materials. Anti-fouling polymer based systems have numerous advantages over conventional materials which include excellent film forming ability, suitable chemical activity, thermal stability, mechanical strength, corrosion resistance, low cost etc. [79].

2.22   Advantages of Polymers as anti-fouling Materials

Anti-fouling polymers have increased efficiency, enhanced anti-microbial behavior, process ability, tunable properties, low toxicity, selectivity, prolonged life time and diverse functionalities. Therefore anti-fouling polymer based systems have given an innovative and new direction in the field of anti-microbial materials. [80, 81]   .

2.23   Factors Effecting Anti-fouling Properties of Polymers Systems

Various factors effect anti-fouling properties of polymers. Surface properties are key parameters to decide the anti-fouling ability of certain material. The environment conditions like pH, temperature, moisture, UV light etc. influence the bio film formation [82–83].Various surface parameters like hydrophilicity [84], surface roughness [85], polarity, surface energy, contact angle etc. are important to design certain anti-fouling system. Baiyan Dong et al. studied the effect of molecular on the anti-fouling properties. Different molecular weight i.e. PEG-200, PEG-400, PEG-600, PEG-2000 and PEG-4800 were grafted onto silicon tetrachloride (SiCl4) plasma functionalized polyethylene terephthalate (PET) surfaces. As the molecular weight of PEG was increased, an improvement in anti-microbial properties of polymer system was observed [86]. Xiangrong Chen et al. correlated the composition and chain length of PEG-PAN copolymer with its anti-fouling properties. The chain length and composition had positive impact on anti-microbial properties of copolymer up to a certain limit [87].

2.24 Classification of Anti-fouling Polymers systems

There are different techniques to modify polymer surfaces for anti-microbial properties. The polymer surfaces may be tuned to bio active or bio passive ones. Bio active surfaces actually kill the fouling organisms on contact through the immobilization of antimicrobial agents. Bio passive surfaces generally consist of hydrophilic well-hydrated polymers which instead repel the biomass and do not allow biotic organisms to deposit on the underlined surface. Another method to classify anti-fouling polymer system is based on either covalent or physical attachment of bioactive molecules. The former, covalent attachment provides better stability, broad range of chemical functionalities and uniformity in comparison with surface physio sorption methods. In covalent attachment, functional bioactive moieties can be attached through a number of ways onto the polymer backbone which include grafting, random or block copolymerization, polymer brushes etc. [88–90].

There are a large number of polymers that have anti-fouling properties. It is important to mention that very less amount of research work has been done on specific anti-algal properties of certain material or polymer because algae has a number of advantages as against its disadvantages and people are using it for beneficial purposes. If we talk about anti-fouling in general, it is linked with anti-bacterial properties. Most of the researchers are working on fouling effects of bacteria or fungus and take algae as a useful microorganism. Although algae have definite advantages it is harmful or hazardous in certain cases.

2.25 Fields Effected by Algal Bio-fouling

In most of the cases the anti-fouling agent which is prone to bacteria has also the capabilities to inhibit the growth of algae or fungus also. Some of the adversely effected fields by micro algae are:

- ✓ Food Preservation
- ✓ Biomedical
- ✓ Water Purity

✔ Photo Bioreactors

✔ Corrosion

✔ Marine Bio-fouling etc.

As an example in photo bioreactors algae sticking along the sides of reactor tubes is a big problem which do not allow sun light to come in. So production of bio diesel stops. The cleaning of tubes is difficult and time consuming because it needs plant shut down. So reactor runs for a short span of time and then automatically stops due to algae sticking alongside tubes. Process becomes low efficient and expensive. Anti-algal systems now play their role by not allowing algae to grow on the side walls which would not only remove the cleaning issues but also improve the efficiency of the process. Polymers can be used in a variety of ways and structures to attain anti-fouling properties.

2.26   Requirements for Anti-fouling Polymer System

2.26.1   Specific Requirements

▪ **Hydrophilicity:** Most of the microbial organisms are hydrophobic, so does micro algae. So any material which is hydrophilic will not allow the sticking or growth of algae. Examples are PEG, Poly dopamine, Poly acryl amide etc. which are all hydrophilic.

▪ **Polar Functional Groups:** There is a strong relationship between hydrophilicity and polarity. Hydrophilic materials generally have polar functional groups. So there is a direct relationship between polarity and hydrophilicity. Examples of some antifouling polymers which having polar functional groups are Poly Ethylene oxide, Poly vinyl alcohol, Poly ethyl amine, Poly methyl meth acrylate etc.

▪ **Lower Surface Energy:** Anti-fouling polymer systems need to have lower surface energy because lower surface energy will not allow surface adhesion or related phenomena. For example polymer brushes are excellent anti-fouling systems which have very low surface energy.

▪ **Lower Contact angle:** Contact angle is directly related to hydrophilicity. Lower contact angle means high hydrophilicity, so anti-fouling polymers should have lower

contact angle which would automatically reduce surface energy and thus surface adhesion of algae. For examples polymer brush PMPC an excellent anti-fouling polymer has contact angle less than 3 degrees.

2.26.2 General Requirements

- **Ease of Processing:** The polymer system to be developed for anti-fouling properties should be easily process able.

- **Raw Material's Availability:** The raw materials should be available in abundance. It is not feasible to go for a polymer which is un abundant and costly.

- **Efficient:** The anti-fouling polymer system should give maximum results as far as inhibition of algae or other microorganism is concerned.

- **Non-Toxic:** The polymer system developed to kill algae should not damage environment and should not be toxic to the people who are dealing with it or to the environment.

- **Economical:** Above all system needs to be economical so that it could be commercialized easily.

2.27　Examples of Anti-Fouling Polymers in literature

- ✓ Poly Ethylene glycol(PEG)
- ✓ Poly ethylene oxide(PEO)
- ✓ Poly Vinyl Acetate(PVA)
- ✓ Poly acryl amide(PAA)
- ✓ Poly methy acrylate(PMA)
- ✓ Poly methyle meth acrylate (PMMA)
- ✓ Poly ethyl enamine (PEE)
- ✓ Poly urethane (PU)
- ✓ Poly glycerol (PG)
- ✓ Poly dopamine(PDA)
- ✓ Polymer Brushes e.g. PVA, PEGMA, PDMAEMA etc.
- ✓ Poly electrolytes like PMPC, PMAPS, PMTAC etc.

2.28   Classification of anti-Fouling Polymer Systems

2.28.1   Polymer Blends

Development of anti-fouling Polyvinylidene fluoride (PVDF) based ultrafiltration membranes using hydrophilic additives like Pluronic F-127, polyvinylpyrrolidone and Poly(ethylene glycol) (PEG). Although current mixtures are sort of composite in which additives act as reinforcement, it can be taken as polymer blend due to two different polymer phases [91]. In one study linear low-density polyethylene was embedded with various biocides and their anti-fungal and anti-algal properties were studied. The biocides used for antifungal properties were Benzimidazol-2-yl-carbamicacid methyl ester [carbendazim (CB)], 5-chloro-2-(2,4 dichlorophenoxy)- phenol [triclosan (TS)] and 3-iodo-2-propynyl N-butyl carbamate [iodopropynyl butyl carbamate (IPBC)]. The antialgal properties were introduced into poly ethylene by induction of 2-methylthio-4-ethylamino-6-tert-butylamino-triazin-1, 3, 5 [terbutryn (TT)] [92].

2.28.2   Co polymers

Block copolymers of siloxane consisting of quaternary ammonium Salt (QAS) groups proved to be excellent anti-bacterial agents. In one study the effect of arrangement of QAS groups in the copolymer chain on antimicrobial activity was studied in which siloxane unit [3-noctyldimethylammoniopropyl] methyl siloxane was bioactive, and dimethyl siloxane acted as neutral unit. The copolymer showed excellent anti-bacterial properties [93].

In a certain study Polystyrene block- poly (ethylene-ran-butylene)-block-polyisoprene is a specific ABC triblock copolymer showing excellent antimicrobial properties. In one such study the triblock copolymer was chemically modified to Semifluorinated-quaternized triblock copolymers (SQTCs). SQTC was then tested against airborne bacterium Staphylococcus via surface analysis which showed about 99 % growth inhibition against given specie [94]. Copolymerization of glycidyl methacrylate and 2-methacryloyloxyethyl Phosphoryl choline (MPC) lead to the synthesis of epoxy based polymer coatings for an oxygen sensing membrane to check the presence of various bio-fouling organisms. The current polymer systems can be used as anti-fouling coating material for the optical bio sensing membranes [95].

2.28.3   Grafted Polymers

Poly ethylene oxide was grafted on poly (2-vinylpyridine) based films. The was actually a specific type of PH responsive 3D grafting. Using such grafted polymers increased the antifouling properties by four times in comparison with conventional surface grafted polymers. This 3D grafting can also be done on other surfaces like nanofiber mats, Nano porous inorganic materials etc. [96]. In one study surface initiated polymerization of 2-hydroxymethyloxirane (glycidol) was done by direct grafting of hyper branched polyglycerol layers on the surfaces of steel, aluminum, and silicon which were oxidized. N-methyl-2-pyrrolidone was used as solvent with heating temperatures between 100 and 140 C°. Current system was at least 90% bio-repulsive against E. coli a specific type of bacterial agent [97]. In a specific research work, the grafting of a zwitterionic polymer, [3-(methacryloylamino) propyl] - dimethyl (3-sulfopropyl) ammonium hydroxide was done on polypropylene non-woven fabric membrane through Oxygen plasma pretreatment and UV-irradiated technique. The resulted polymer system provided with decreased protein adsorption & platelet adhesion and improved flux recovery up to 90% [98].

2.28.4   Polymer Coatings

Studies have found that various ionic and non-ionic natural rubber-based coatings have capability to inhibit the growth of microbial organisms like bacteria, fungi, microalgae etc. In one such study the anti-algal properties of various rubber based coatings like Acrylate cis-1, 4-polyisoprene cationomer (acrylate PI cationomer), diacrylate cis-1, 4-polyisoprene (diacrylate PI and epoxidized hydroxytelechelic cis-1,4-polyisoprene (EHTPI) were studied.   The impacts of these coatings on marine micro algal photosynthesis was examined using pulse-amplitude-modulation (PAM) fluorescence method. The growth inhibition in the range 44% to 100% was observed depending on the algal species, the respective rubber coating and its ionic strength [99].

Poly dopamine and poly (N-vinyl pyrrolidone) have excellent anti-fouling abilities. When both are used together, the results are even fascinating. In one such study a typical polypropylene porous membrane was first dip coated with PDA layer and then by poly (N-vinyl pyrrolidone) (PVP) layer.   There were multiple hydrogen-bonding based interactions between PDA and PVP giving a very strong anti-fouling material. Then water

hydrophilicity and wettability of the membranes was examined using contact angle measurements. There was a significant improvement in hydrophilicity and wettability. Oil/water emulsion filtration, protein filtration, and adsorption tests showed remarkable antimicrobial activity [100]. In a specific research work, Triblock polymer coating consisting of poly ethylene glycol, cationic polycarbonate, and adhesive functional block showed excellent anti-microbial properties [101]. Fluoropolymer, fluorinated and non-fluorinated siloxane based coatings were deposited on stainless steel surfaces using the atmospheric plasma jet system known as Plasma Stream™. Fluoro siloxane and fluoropolymer coated steel instruments showed a significant reduction in bio fouling [102].

2.28.5   Thin Films

Oligo Ethylene glycol based self-assembled monolayers of alkane thiolates were deposited on gold in one study. The resultant anti-fouling system provided excellent results [103].

2.28.6 Poly Electrolytes

In an important research work carried out by Motoyasu Kobayashi et al. the surface properties like wettability, contact angle and surface energies were calculated for various poly electrolytes and polymer brushes. These properties were then linked with anti-fouling behavior of materials. Initially different types of polymer brushes having ionic and nonionic functional groups were deposited on silicon substrates by surface initiated atom transfer radical Polymerization. Static and dynamic contact angle measurements were made using water and hexadecane. These measurements were made both in air and in water using captive bubble measurements. Surface free energies of various samples were also calculated using Owens-Wendt equation. There were two set of depositions namely poly electrolytes and polymer brushes. Polymer brushes showed relatively higher values of surface energy and contact angle in comparison with poly electrolytes which had very low surface properties. The lower surface properties in poly electrolytes are attributed to ionic nature of functional groups in them. In any case both of them showed excellent self-cleaning abilities and anti-fouling properties [104].

2.28.7 Polymer Brushes

In a research article amphiphilic polymer brushes were made on polypropylene nonwoven fibers and hydrophobic self-assembled n-octadecyltrichlorosilane (ODTS) monolayer which was taken as reference. The assemblies were made by a three-step functionalization process. In the first step, denatured proteins were adsorbed on respective PP and ODTS Surfaces. Then cross-linking of both surfaces was done via glutaraldehyde using sodium borohydride (NaBH4). In the last step terminal hydroxyls of HEMA's pendent groups were modified with fluorinating moieties of different chain lengths, which resulted in amphiphilic brushes which showed excellent activity against microbial organisms [105]. In another similar work Zwitterionic based polymers brushes, poly (lysine meth acrylamide) and poly (ornithine meth acrylamide) were grafted on gold through surface initiated photoiniferter-mediated polymerization. The grafted polymer brushes show extremely low bio-fouling which can be used for wide range of applications like implant coating, drug delivery, bio sensing etc. [106].

2.29 Subject Approach

In Subject case, Poly(ethylene glycol) (PEG-1000) was selected as anti-fouling agent in general and anti-algal agent in specific. PEG was blended with Polystyrene in different concentrations ranging from 5% to 20% synthesizing various blends. Besides PEG, Silver sulfadiazine was also used as anti-algal agent in 2 of the blends. So there were four PEG based blends and two Silver sulfadiazine based blends. All of the samples were blended using extrusion plastometer i.e. solid state processing technique. The reason behind using PEG is that it is not only excellent anti-fouling agent but has other advantages as well such as:

- PEG has been used in variety of forms like blends, copolymers, grafting, thin film, coating etc. with other polymers. It is also used in combination with nano particles of other material to induce anti-microbial effects.
- It is worked on by a number of researchers.
- The blending method with Polystyrene is quite simple.

- It is easily available and economical as well.

2.30   Poly (ethylene glycol)(PEG)

Poly (ethylene glycol)(PEG) is a poly ether obtained by addition polymerization of ethylene oxide and water. It is basically a linear polymer possessing distinct chemical profile making it the beneficial product as far as biological, chemical and pharmaceutical applications are concerned. PEG-100 is a grade of Poly (ethylene glycol)having molecular weight 1000 units. It is a low melting waxy solid with melting point between 35C to 40C. This is a typical grade to be used as anti-algal agent in present project.

2.31   Physical & Chemical Properties

- **Chemical Properties:** The general structural formula of PEG is $HOCH_2 - (CH_2 - O - CH_2)_n - CH_2OH$. Its chemical formula is $(C_2H_4O)_{n+1}H_2O$, where n is the repeating unit indicating number of oxyethylene groups. The formula weight is in the range 200-9500 units [107].

- **Molecular weight & Physical State:** The low molecular weight polymers (less than 700 molecular weight unit) are mostly in viscous liquid state. These are generally little hazy, colorless, odorless and hygroscopic. Those between 700 and 900 are semi-solids. As the molecular weight of PEG is increased above 1000 units they become creamy white waxy solids, flakes, or free-flowing powders depending upon their molecular weight.

- **Solubility:** PEG compounds are mostly water soluble. They are also soluble in a number of organic solvents mostly aromatic hydrocarbons like chloroform, glycol ethers, esters, aliphatic ketones and alcohols. Their solubility in aliphatic hydrocarbons is minimum. As the molecular weight of PEG increases their solubility in respective solvent decreases. Viscosity is mostly molecular weight dependent which increases with increase in molecular weight.

- **Hygroscopic:** PEGs have ability to attract and retain moisture from the atmosphere being hygroscopic. Their moisture retention ability decreases with molecular weight.

- **Viscosity:** Viscosity of PEG grades increase with increase in temperature.
- **Stability:** PEGs are thermally stable polymers with low volatility. The thermal stability increases with molecular weight [108]. Table (2) highlights some physical properties of PEG-1000 in numbers [109].

Table 2: Physical Properties of PEG-1000

Physical Property	Value
Appearance	White Paste
Density at 60C° (g/cm3)	1.0927
Melting Point (C°)	35 – 40
Water Solubility at 20°C(% by wt)	80
Water Content	0.5 Max
Viscosity at 100°C (cSt)	17.2
Specific Heat (cal/g/ C°)	0.51
Heat of Fusion (Cal/g)	38
PH at 25C°	4.5-7.5
Flash Point (Closed Cup, C°)	177
Flash Point (Open Cup, C°)	277

2.32   Typical Applications

PEG and its various grades have wide variety of applications. Some of the general applications include

- ✓ Adhesives
- ✓ Ceramic Glaze
- ✓ Chemical Intermediates
- ✓ Food Packaging

- ✓ Lubricant
- ✓ Mold Release Agent
- ✓ Wood Treatment etc.

2.33   Antifouling Phenomena of PEG

The anti-fouling or anti-algal phenomena is directly linked to the surface properties and polarity of certain material. Antifouling Phenomena of PEG is attributed to the following factors.

- **Hydrophilicity:** PEG is hydrophilic while most of the microbial organisms are hydrophobic, so it would automatically repel the fouling species. According to literature survey hydrophilicity is directly associated with antifouling performance.
- **Steric Repulsion:** Poly (ethylene glycol)(PEG) undergoes steric repulsion phenomena, due to its long chains which don't allow certain organism to settle or grow on its surface. This mechanism is general to polymers having long chains.
- **Polar Functional Groups:** PEG got high polarity due to presence of OH group which also leads to hydrogen bonding. This high polarity is directly linked with hydrophilicity which inhibit the growth of certain bio organism.
- **Lower Contact angle:** The contact angle of PEG is 63° which is lower than a number of other polymers. This lower contact angle is also associated with high magnitude of hydrophilicity against the microbial organisms which are hydrophobic with higher contact angles. This difference in wetting and adhesion abilities don't allow settling of any fouling agent.
- **Surface Energy:** Critical Surface Tension value for Poly (ethylene glycol)is 43 units. This lower energy means lower chances of adhesion by some foreign organism [110].

2.34   Application of PEG as Anti-fouling Material

Poly(ethylene glycol) (PEG) hydrogel along with cell adhesive polypeptide is used for wound healing applications [111]. Oligo (ethylene glycol)-Terminated Self-Assembled monolayers of alkane thiolates show strong bacterial resistance on gold substrates [112]. PEG can also be used as stimuli responsive anti-fouling material. In one such example,

3D grafting of Poly(ethylene glycol) was done on PH-stimulative poly (2-vinylpyridine) films. As a result there was a 4 times improvement in antifouling behavior in comparison with the surface grafted polymer [113].

PEG based Hydrogel polymer systems are used for antimicrobial applications in freshwater and marine based Environments [114]. Triblock polycarbonate based polymers have potential to inhibit catheter based bloodstream infections. These anti-fouling systems consist of three components namely, Poly(ethylene glycol) , cationic polycarbonate, and an adhesive functional block [115]. Thin film based composite membranes made up of Silver PEGylated dendrimer nanocomposite are used for water treatment against bio fouling [116]. There is a new class of anti-microbial ultrafiltration membranes synthesized of polyvinylidene fluoride embedded with PEGylated polymeric particles used for water treatment [117]. PEG based coatings synthesized by atmospheric plasma have excellent protein and cell repulsion capabilities [118].

2.35   PEG specific Blends

 PEG can be blended with a wide variety of polymers. In present case it is blended with Polystyrene for anti-algal applications. It can be blended with other polymers for a variety of novel applications. For example it can be blended with poly vinyl chloride used for lithium polymer cells [119]. Poly (ethylene glycol) is used as a thermal energy storage material in combination with polyoxymethylene and Poly vinyl chloride [120].

2.36   Polystyrene(PS)

Polystyrene also abbreviated as PS is a thermoplastic with a long hydrocarbon chain and phenyl group attached to every alternative carbon atom. It is basically vinyl based aromatic hydrocarbon. The chemical formula is C_8H_8 and IUPAC name is Poly (1-phenylethene). It is relatively inexpensive, hard, stiff and transparent resin used in variety of fields like food packaging, laboratory ware, insulation, protective packaging, surfboards, automobile parts etc. [121-122].

2.37   Polystyrene Types

Polystyrene is available in various forms like isotactic, sydiotactic and atactic types.

- **Isotactic:** It is a semi-crystalline form of Polystyrene.
- **Syndiotactic**: In Syndiotactic Polystyrene, phenyl groups are positioned on the alternating sides of carbon backbone. This allows perfect alignment and thus packing of carbon chains inducing high crystallinity.
- **Atactic:** Atactic is the most important commercial form of Polystyrene practically used in most of its applications. There is a random distribution of phenyl groups on both sides of the carbon chain which prevents the alignment of carbon chains failing to provide sufficient regularity to achieve any crystalline structure [123].

2.38   Physical & Chemical Properties of Polystyrene

Polystyrene is highly transparent due to the presence of large aromatic ring type functional groups, which do not allow packing of polymer chains properly thus reducing the crystalline arrangements. The rigidity of polymer chains is also due to these aromatic rings which inhibit the rotation of the chains around the carbon-carbon back bone. It has an intermediate melting point and is a poor barrier against oxygen and water vapor [124]. It is chemically quite inert. It is highly flammable. PS is soluble in some organic solvents like DMSO, Toluene, Acetone, Benzene and THF but insoluble in water. Polystyrene is not entirely water or moisture proof in any of its form. Table (3-4) list the general physical, mechanical and electrical properties of Polystyrene respectively [125-127].

Table 3: Physical Properties of Polystyrene

Properties	Value
Water Absorption (%)	0.03-0.1
Density (g/cm^3)	0.95–1.03
Melting point (C°)	240-270
Glass Transition Temperature(C°)	90-100
Thermal conductivity W/(m·K)	0.033
Refractive index(n_D)	1.6
Specific Heat Capacity (J/Kg-K)	1250
Thermal conductivity(W/m-K)	0.14
Water Absorbtion (%)	0.03-0.1

Table 4: Mechanical & Electrical Properties of Polystyrene

Mechanical Property	Value	Electrical Property	Value
Elastic Modulus (MPa)	3000-3600	Dielectric Strength (MV/m)	20
Elongation (%)	1 to 5	Dielectric Constant	>10^{16}
Tensile Strength(MPa)	30-60	Volume Resistivity (ohm-cm)	2.5
Hardness(Rockwell Scale)	M45-M60	Arc Resistance (sec)	70
Impact Strength (J/m)	37-59		
Flexural Strength (MPa)	76		

2.39 Typical Applications of Polystyrene

- ✓ Model cars and airplanes are mostly made up of Polystyrene.
- ✓ PS is used as insulation in various products like refrigerators, freezers building walls and roofing etc.
- ✓ It is also used for packaging purposes of various appliances.
- ✓ Disposable plates, drinking cups, rigid trays & containers, eating utensils, bowls etc. used in food-service industry are made of Polystyrene.
- ✓ Blends and copolymers of Polystyrene provide rigidity and hardness to soft plastics and rubber products.
- ✓ Various electrical home appliances like air conditioners, microwave ovens, Juicers, blenders, refrigerators, vacuum cleaners etc. are mostly made up of Polystyrene and its blends due to its inertness and cost effectiveness.
- ✓ A number of car parts including instrument panels, knobs, trim, door panels etc. are made of is used to make many car parts, including.
- ✓ Some pharmaceutical and medical instruments are also made up of Polystyrene.
- ✓ Polystyrene is used in manufacturing of windows, lenses, gauges glasses, indicators & dials of automobiles utilizing its optical properties [128].

2.40 Silver sulfadiazine

Silver sulfadiazine is an anti-fouling agent used in medicine as a topical cream on burns. It is basically an organic compound belonging to the family of Benzene and substituted derivatives. It has a very broad anti-fouling effect against both the gram positive and gram negative bacteria.

It has exceptionally well anti-fungal properties as well. It is also quite effective against yeast and micro-algae [129-131]. The anti-fouling activity is due to the synergetic effect of both silver & sulfadiazine It is generally available in the form of aqueous suspension or cream.

2.41 Physical Properties

The IUPAC name is silver (4-aminophenyl)sulfonyl-pyrimidin-2-ylazanide with the molecular formula C10H9AgN4O2S. The molecular weight is 357.13726 g/mol. The melting point is around 285 °C. It is water soluble with the solubility of 7.87 mg/mL. Fig (1) shows the structure of Silver sulfadiazine.
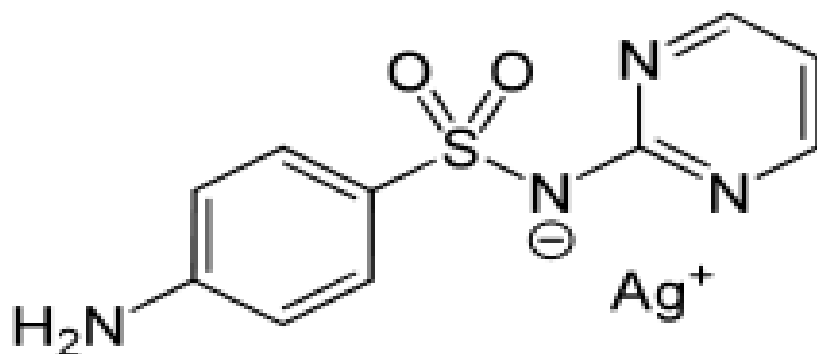
Fig 1: Chemical Structure of Silver sulfadiazine

Chapter 3

MATERIALS & METHODS

3.1 Materials & Reagents

The details of various materials and reagents used in current research study are listed and explained below.

1) Poly(ethylene glycol) (PEG-1000)
2) Polystyrene(PS)
3) Silver sulphadiazine(SS)
4) Dimethyl sulfoxide(DMSO)
5) Distilled Water
6) Mueller Hington Agarose (MHA)
7) Bold Basal Medium(BBM)
8) Absolute Ethanol
9) Spirit lamp
10) Glassware
11) Algal strain: Dictyosphaerium sp. strain HM1 (DHM1)
12) Algal strain: Dictyosphaerium sp. strain HM2 (DHM2)
13) Algal strain: Pectinodesmus sp. strain HM3 (PHM3)
14) Paper Disks
15) Aluminum foil
16) Sterile Cotton wool swabs
17) Sterile Petri dishes
18) Forceps, Pippete & Algae spreader
19) Safety Gloves, Face Masks, Lab Coat etc.

3.1.1 Details about Reagents

Poly (ethylene glycol)(PEG-1000) was purchased from Dae Jung Chemicals & Metals Co, Ltd and used without further pretreatment. Polystyrene (SS) was obtained from Sigma

Aldrich and utilized without further modification. Silver sulfadiazine (SS) was supplied by Sigma Aldrich. Various algal strains namely Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3) were kindly provided by Atta-Ur-Rahman School of Applied Bio Sciences (ASAB), NUST Islamabad. BBM (Bold Basal Medium) was courteously supplied by Centre for Energy System (CES) NUST, Islamabad. Mueller Hington Agarose (MHA) for analytical electrophoresis with Electrodosmosis (0.05-0.13) and Gel point (34-38C°) was purchased from Merk Private Ltd. DMSO (Dimethyl sulfoxide) was obtained from Sigma Aldrich. Other reagents were AR grade and used without further purification.

3.2 Equipment & Characterization Tools

The following instruments and characterization equipment were used for material's synthesis and characterization. The details about respective model and specifications are also given along with each characterization tool.

1) Extrusion Plastometer
2) Heavy Blender
3) UV Safety Cabin
4) Auto Clave Machine
5) Universal Testing Machine (SHIMADZO 20KN)
6) Optical Microscope (OPTIKA 600, B-600 MET)
7) TGA Equipment (TGA/DSC with High Temperature Furnace Metler Toledo)
8) DSC Equipment (DSC Q20 with RCS 90, TA instruments)
9) UV Visible Spectrophotometer(UV-2800 Spectrophotometer Scientific Tech. Corporation (PVT) LTD)
10) Zetasizer (Zetasizer nano zsp Malvern instruments Ltd)
11) Dilatometer (Dil 2012 std, ORTON Dilatometer, S type Thermocouple)

3.3 Sample Preparation

The samples were prepared using Extrusion Plastometer. The polymers, Polystyrene (PS) and Poly (ethylene glycol) (PEG-1000) as mentioned above were used to form various blends. PEG in the form of sticky powder was used in as received form while PS was meshed using Blender. Powder size of PS was less 500 mesh size. Both Polystyrene and PEG are thermoplastics. Extrusion Plastometer is an instrument used to find MFI of thermoplastics. Using MFI we can guess about polymer's viscosity and molecular weight etc. Plastometer works in a similar way to extrusion which is among the excellent methods employed in polymer processing especially thermoplastics.

The temperature of the equipment was set to $200C^o$ using display buttons and at the same time dye was placed in the barrel. The dye was fitted so that it was well stuck at the bottom of the barrel. The equipment was allowed to preheat for almost 5 minutes to allow the temperature to be stabilized. Meanwhile the plunger weighing 5kg was also placed in the barrel for same purpose of preheating. The powders to be blended were mixed adequately before usage. PS and PEG in specific concentrations were then placed in plastometer. Once the powder gets heated apply plunger in the equipment. As the heavy plunger moves on, it squeezes and mixes the powders, which under heavy pressure and high temperature melts the underlined materials. The melted materials passes through the dye which has small hole of 3 mm diameter in it. As the process continues the end product in the form of long cylindrical rod comes out. The rod is of the same diameter as the dye hole. In this way whole of the powder in the form of cylindrical rod is obtained as output. Once the process is complete and there is no powder coming out, pull out the dye when it is hot, because it's only possible to clean it while in heated condition. Clean the dye and equipment at the end. Care should be taken when removing dye as it's hot and may damage your skin, so perform the process using proper gloves and face masks to prevent fumes coming out as the material gets hot. All of the process was done according to ASTM standards (D1238-13) for MFI of Polystyrene. Fig (2) highlights the various steps during sample preparation.
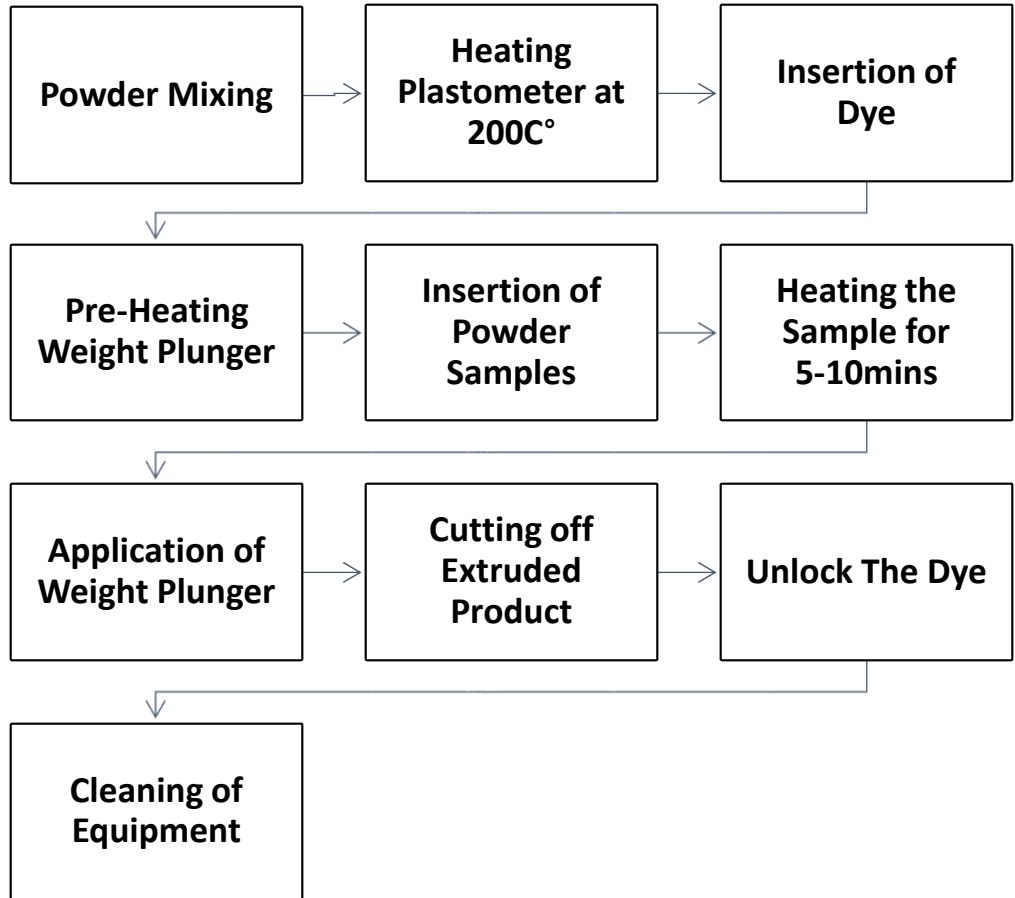
Fig2: Flow diagram of Sample Preparation through Extrusion Plastometer

In present case each sample weighing 2g was used. The powder samples were named as S0, Sa, Sb, Sc, Sd, Se and Sf. The samples vary with respect to the type and concentration of powders. The different powders like PEG, PS and Silver sulphadiazine (SS) were used according to weight percentage. For example S0 is pure Polystyrene weighing 2g while Sf contains 10%PEG, 10%Silver sulphadiazine & 80% PS with the total weighing 2g. The complete details of samples are given in following table (5).

Table 5: Compositional Details of various Samples

Sr. No.	Sample Designation	Sample Description
1.	S0	Pure PS
2.	Sa	5%PEG/PS Blend
3.	Sb	10%PEG/PS Blend
4.	Sc	15%PEG/PS Blend
5.	Sd	20%PEG/PS Blend
6.	Se	10%SS/PS Blend
7.	Sf	10%PEG/10%SS/PS Blend

3.4 Characterization Techniques

3.4.1 Agar disk Diffusion Method

After the polymer blends were synthesized, the next and the most important step was to study their antifouling properties in general and anti-algal properties in specific. Three different types of algal strains were employed to measure anti-algal behavior namely Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3). There are qualitative as well as quantitate methods available to study anti-algal characteristics. As a simple qualitative method and to guess the behavior of PEG in general all of the three algal strains were put in to vials (small bottles). About 10ml of algal strains were put in to the bottles. Then PEG and all of the blends were put into the bottles. The concentration of each sample was 2g in 10 ml. Then all of the samples were placed on shaker where samples were put under constant vibration for 1 week. The samples containing PEG were colored brownish or lightly brownish after one week which were light green initially. This was a clear indication of something happening to algae. The color change is a proof of algae been killed by respective samples containing anti-algal reagent PEG.

The complete detailed steps of Agar disk Diffusion Method are discussed below.

I. For one experiment a total of 6 petri dishes were required, 3 for antialgal study of PEG and 3 for anti-algal studies of polymer blends. A total of three petri dishes with

respect to every algal strain were used in every case. The strains for which anti-algal study is performed are already mentioned in previous sections.

II. Prepare 1% Agar solution using 1g agarose in 100ml BBM solution in conical flask i.e. for 100ml BBM solution containing 80ml distilled water and 20ml BBM media (total 100ml solution), 1g agarose is required. For 6 petri dishes almost 250ml solution is required which is more than enough. Autoclave agar solution including all of the accessories used in Disk diffusion method including petri dishes, forceps, pipette, algae spreader etc. Before operating autoclave equipment make sure that exhaust of machine is closed and water is up to the mark. If not then put some distilled water. The accessories like forceps, petri dishes etc. should be wrapped in aluminum foil before putting them in equipment. The complete autoclave cycle would require almost 2 hours. The purpose of autoclave is to sterilize the equipment and accessories**.** Agar solution dries very quickly, so try to use it at earliest, otherwise if once dried it's impossible to pore it or empty the flask.

III. The pouring of agar solution in to the petri dishes, algae spreading and all associated phenomena mentioned below needs to be done in bio safety cabin. Before pouring solution into the autoclaved plates switch on the UV light for 10 to 15 minutes and close the cabin. Stay away from the cabin when ultraviolet light is on because such light can severely damage your health. This UV light need to be switched on almost 20 to 30 minutes before the completion of autoclave because the solution may dry within minutes so caution should be taken. The purpose of UV is to make the working cabin clean. The cabin should be as clean as possible because even minute amount of contamination can affect the microbial growth and thus disturb the expected results. After 10 to 15 minutes switch off the UV lamp. Spray absolute ethanol in the cabin and clean it with tissue papers or any other stuff like that.

IV. Switch on the spirit lamp. The whole process needs to be done under spirit lamp to avoid any other undesired microorganisms and contamination except microalgae. Under the lamp pour the agar solution in petri plates, so that it completely fills the plate with almost 2mm thick layer roughly. Repeat this process with all of the dishes. Keep the dishes slight open for 15 to 20 minutes to dry the agar solution in the plates and formation of agar layers.

V.   Before spreading algal strains on petri plates, make dilutions or suspensions of some solvent, like DMSO in present case. The solvent needs to be non-anti-fouling. All of the different types of polymer blends in different amounts were dissolved in specific amounts of DMSO separately. A total of 13 different dilutions were made and placed in small bottles. Then sterilized filter paper disks were put in to the dilutions, so that they attach the specific amount of material. The dilutions were then stored in a cooler place until being used. The complete details of dilutions are given in the following table (6).

Table 6: Detail of Various dilutions used in Disk Diffusion Method

Dilution Name	Sample Weight	Description of Dilution
S0	2g	Pure PS in 20ml DMSO solution
Sa	2g	5% PEG/ PS blend in 20ml DMSO solution
Sb	2g	10% PEG/ PS blend in 20ml DMSO solution
Sc	2g	15% PEG/ PS blend in 20ml DMSO solution
Sd	2g	20% PEG/ PS blend in blend 20ml DMSO solution
Se	2g	10% SS/ PS blend in 20ml DMSO solution
Sf	2g	10% SS/ 10%PEG/ PS blend in 20ml DMSO solution
S1	0.05g	0.05g PEG in 15ml Distilled Water
S2	0.1g	0.1g PEG in 15ml Distilled Water
S3	0.15g	0.15g PEG in 15ml Distilled Water
S4	0.2g	0.2g PEG in 15ml Distilled Water
S5	0.1g	0.1g Silversulphadiazine in 15ml Distilled Water
S6	0.2g	0.2g (1g PEG + 1g SS) in 15ml Distilled Water

VI.   The next process is application of micro-algal strains on to the petri dishes. It is necessary to have sufficient quantity of algal strain in its respective solution, so that it can disperse uniformly across the plate. The as received algal solutions were not concentrated enough, so they were undergone through centrifugation at 5000rpm for

15 minutes. The obtained algal dilution after decanting off the clear solvent was highly concentrated. 5ml solution of every strain was more than enough. Algae spreading was again performed in bio safety cabin using the same protocol as applied in agar solution dispersion under extremely clean environment. Algal sample of every type was dropped on petri plates using pipette. The spreader was used to fully disperse the solution uniformly. The whole process was done under spirit lamp.

VII.   Once the specific type of strain was dispersed, spreader was washed with ethanol and dried using spirit lamp, so that this present algal strain doesn't grow in to the second plate and so on. After all the three types of algal strains were uniformly dispersed, the next process was to check the anti-algal activity of blends. Paper disks of every blend from respective dilution was placed on the petri plate at specific place. The specific areas corresponding to each type of blend were already spotted on by marker. For example for PS blends, 6 equal portions were made on the petri plate corresponding to each type of blend namely Sa, Sb, Sc, Sd, Se and Sf. The disks were placed at the respective areas on petri plates. The control disk was also placed at the Centre. The control disk in actually of solvent, DMSO in present case, which needs not to be antialgal. Otherwise the antialgal activity of samples cannot be judged. DMSO and distilled water did not show any antialgal activity at all. The plates were then placed under fluorescent light for 3 to 4 days, or even weak to see the anti-algal behavior of various samples.

VIII. After 3 to 4 days different zones of inhibition were observed for every blend except pure Polystyrene, distilled water and DMSO solvent. The zone of inhibition was measured to calculate antialgal activity. The process was repeated 4 to 5 times to get efficient results using other solvents also like Toluene and THF which proved to be anti-algal, so were not practically helpful.

The inhibition of algal growth through disk diffusion method corresponding to various blends was calculated using following relations.

a.   **IAD (a) = (d_{clear}- $d_{specimen}$)/2**

Where IAD (a) is Inhibition of Algal growth Factor with respect to diameter

d_{clear} is diameter of algal inhibition zone

$d_{specimen}$ is diameter of disk excluding algal inhibition zone

b. IAD (b) = (A2-A1)

Where IAD (a) is Inhibition of Algal growth Factor with respect to area

A2 is area of algal inhibition zone

A1 is area of disk excluding algal inhibition zone

The complete protocol of Agar disk Diffusion Method is highlighted in fig (3)

Prepare Dilutions of various Samples & put Sterilize Paper disks in them
Prepare 2% Agarose Solution in distilled water & BBM Media
Mark Sample names & Design separate portions for Anti-algal activity of every Sample
Autoclave Agar Solution, Petri-Plates, Forceps, Algae Spreader, Pipette etc.
Sterilize Bio safety Cabin through UV light & clean it using Absolute Ethanol
Pour Autoclaved Agar solution in Petri-plates under Spirit lamp in Safety Cabinet
Centrifuge Micro-algae at 5000rpm for 15 mins & get 2ml -5ml Concentrated Solution
Let the Agar solution Dry in Petri-dishes
Spread Microalgae in Petri plates using Pippete & Algae spreader under Spirit lamp
Place Paper disks on the respective marked portion of every Sample
Place the Petri-plates continuously under Fluorescent light for 3-4 days
Measure diameter of Inhibition zone and calculate IAD factors for every Sample

Fig 3: Flow chart explaining different steps of Disk diffusion Method

3.4.2 Mechanical Testing

Tensile testing of specimens was performed using Universal Testing Machine (SHIMADZO AG-X PLUS 20KN) especially designed for polymers. The ASTM standard D638-14 was used to find the mechanical properties of rigid rods as the samples were in

the shape of solid rods. The main focus was on tensile properties especially elongation at break, tensile strength and elastic modulus obtained from resultant graphs.

Sample Preparation: Seven different types of samples namely S0, Sa, Sb, Sc, Sd, Se and Sf were mechanically tested whose details are already given in previous section. The length of specimens was 10cm and diameter 4mm each. Gripped section was 20mm from each end. Remaining 60mm was gauge length. The extension rate was 1mm/min due to the samples being extremely thin and higher strain rate could produce falsified and ambiguous results.

The sample was gripped between the jaws of the equipment. The adjustment and tightening was at minimum level because samples were polymer thin rods instead of metallic pieces, otherwise sample may break even before experiment. This is simply trial and error method. The method and test files including different parameters related to samples were created in the software. Once gripped firmly adjust all of the initial load and strain to zero before starting the test. Once parameters set to zero, just start the test. The stress/strain curve will be drawn automatically. The experiment will stop as the sample is broken, which breaks at the weakest point within the gauge length. Remember UTM graphs of polymer samples may not be as smooth and clear as metallic samples. Remove the broken pieces from machine and add a new one. Repeat the same process for all the samples.

3.4.3 UV-Visible Spectroscopy

UV-Visible Spectroscopy of samples was done using UV-Visible Spectrophotometer. The apparatus specifications were UV-2800 Spectrophotometer Scientific Tech. Corporation (PVT) LTD. The instrument can calculate us a number of important parameters but the purpose of following experiment was to merely check the absorbance of different samples against UV Visible spectrum and analyze the anti-algal behavior. The sample on which algae is grown uniformly across would give high absorbance as light will not find way to escape out. As the amount of anti-algal agent increases, algae growth would decrease resulting in an increased area from which light can escape. As a result absorbance will increase as their will be no obstruction on the way of light. So as the anti-algal content

increases, absorbance decreases. This experiment cannot only reanalyze the results of Disk diffusion method but also give an idea about antifouling and transmittance abilities of the blends if they are to be used for photo bioreactors Scanning was done from 300nm to 800nm including ultraviolet and visible regions. Sampling interval was 1nm and scan speed was set to middle. Reference Test was performed once.

Sample Preparation: For this experiment glass slides are needed. Sample preparation is done using the protocol of disk diffusion method. If all of the three types of algal strains are were used for all of the samples, then a total of 21 samples would have been produced (7 for each algal strain). Due to testing limitations a total of 7 samples were used. The samples were same S0, Sa, Sb, Sc, Sd, Se and Sf. The first three samples (S0, Sa & Sb) were tested against Dictyosphaerium sp. strain HM1 (DHM1), the other two (Sc & Sd) against Dictyosphaerium sp. strain HM2 (DHM2) and remaining two (Se & Sf) against Pectinodesmus sp. strain HM3 (PHM3). The protocol for algal growth was same as used in agar disk diffusion method discussed already in previous sections. The only difference now is that algae was deposited on glass slides. More care was taken to deposit uniform films of agar and microalgae using pipette on glass slides. The process until now was same for both cases except the more complexity in later one. The dilutions of different polymer blends in DMSO were already prepared before. Once the micro-algal layer was grown, the next step was to deposit the layer of anti-algal compounds. The same was done using pipette. All of the different dilutions were deposited on glass slides to study their effect on algal growth. The glass slides were placed in petri plates during above depositions. After the above processes petri dishes were placed under fluorescent light for 3 to 4 days. After the specific time when algae was grown on the plates, the samples were ready to be analyzed further.

The samples were then undergone through UV-Visible spectroscopy. Once the reference scan was done, then all of the samples were put in to the apparatus one by one and tested against reference which was automatically set to zero by the apparatus. It took almost 5 minutes to scan a single sample completely across the spectrum 300nm-800nm. The whole experiment was done in less than 1 hour. Care should be taken while mounting and releasing the samples, so that it may not damage the sensitive equipment. The spectrums were obtained which were finally analyzed.

3.4.4 Thermogravimetric Analysis

Thermogravimetric Analysis (TGA) was done from Institute of Space Technology (IST) Islamabad. The instrument specifications were TGA/DSC 1 with High Temperature Furnace Metler Toledo. The purpose was to find the weight loss of the polymer blends with temperature and find their thermal stability and guess about their degradation. Samples were scanned until 800C° under Nitrogen atmosphere using scan rate of 10 C° /minute. The single sample was experimented within 20 to 30minutes. Finally a curve was plotted between temperature and heat flow.

Sample Preparation: For TGA a very minute amount of sample is required which is in mg. The sample should preferably be in powder form because the sample pan is extremely small and in powder form it is easier to put the sample in. The samples in subject case were rigid rods with extreme difficulty or impossibility to get a small enough piece out of it to be used in experiment. The samples were thus converted in to powder form. Samples were crushed manually to get an adequate powder size. Meshing was done manually. It can also be done through blender if sample is in access quantity. Thus all of the samples were crushed to fine enough powder to be used in apparatus. TGA was done to obtain various plots corresponding to every sample and then analyzed. The graphs were automatically plotted between temperature and weight loss.

3.4.5 Differential Scanning Calorimetry

 Differential Scanning calorimetry abbreviated as DSC was done to find out an important thermal transition i.e. Glass Transition Temperature (T_m). Although DSC can be used to find out numerous parameters like degree of crystallization, heat capacity, enthalpy of fusion but we mainly focused on thermal transitions and associated temperatures which can also give an idea about how well dispersed different component phases are within a blend.

Sample Preparation: For DSC just like TGA, a very minute amount of sample is required which is in milligrams. The sample should preferably be in powder form because the sample pan is extremely small and in powder form it is easier to put the sample in. The

samples in subject case were rigid rods with extreme difficulty or impossibility to get a small enough piece out of it to be used in experiment. The samples thus needed to be converted in to powder form. Samples were crushed manually to get an adequate powder size. Thus all of the samples were crushed to fine enough powder to be used in apparatus.

The apparatus used to perform experiment was DSC Q20 with RCS 90, TA instruments. The characterization was done from Institute of Space Technology (IST) Islamabad. The weight of each sample in mg was measured in advance so that it could be used further in calculations. Samples were scanned until $250C^o$ under Nitrogen atmosphere using scan rate of $10\,C^o$/minute. The single sample was experimented within 20 to 30minutes. Finally a curve was plotted between temperature and heat flow. The results were then further analyzed to find various parameters.

3.4.6 Zeta Potential Measurement

Zeta potential measurement was done against all of the three different types of algal strains namely Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3). The specific instrument was Zetasizer nano zsp Malvern instruments Ltd.

The equipment can measure a variety of factors like:

- ✓ Zeta Potential
- ✓ Size of colloidal dispersion of particles in range 0.1 - 1000 nm
- ✓ Poly dispersity Index
- ✓ Conductivity of colloidal dispersion
- ✓ Mobility of colloidal Particles etc.

But the main focus was measurement of zeta potential and particle size of different algal strains. Electric field exists in present equipment which measures Zeta potential. There is an established fact that like charges repel and unlike charges attract each other. The ions as a result of electric field will attract or repel the suspended particles. Ions close to the surface of the particle, will be strongly bound while ions that are further away will be

loosely bound forming a layer named diffuse layer. Within the diffuse layer there is a certain boundary. Ions present within this boundary will move with the particle when it moves in the liquid. The ions outside the boundary will stay where they are. This boundary is called the Slipping plane. The potential of this slipping plane is actually called the Zeta potential. This potential depends on distance from the particle surface.

Zeta potential is measured using a combination of the measurement techniques like Electrophoresis and Laser Doppler Velocimetry. The equipment measures the velocity of a particle when it moves in a dispersing liquid. Once the velocity of the particle and the applied electrical field is known, we can work out the zeta potential, by using other two known parameters of the sample viscosity and dielectric constant,. All of this calculation is done by the equipment which directly gives us Zeta potential value. The basic principal behind particle size measurement is to use Dynamic Light Scattering (DLS) by correlating with Brownian motion of colloidal particles and determine the hydrodynamic size. Similarly other parameters are also calculated by the equipment automatically. In present case suspension of algal strains was made in distilled water, then their zeta potential and other parameters were calculated using the equipment.

3.4.7 Optical Microscopy

Optical Microscopy was done to prove the results of agar disk diffusion method. The experiment was done using OPTIKA 600, B-600 MET. The cultured petri plates of each type obtained after disk diffusion method were analyzed under microscope. There were a total of 6 plates (2 for each strain type). Three of them had eight samples each while three had six samples each containing various samples. Each sample was analyzed under microscope using absorbance mode because reflectance mode was not practically possible due to geometry of plates. Under the microscope, the boundary area between inhibition zone and algal growth was imaged. There was a clear contrast against various anti-algal agents and blends. The area containing algae didn't allow the light to pass on, absorbing all the light and was thus imaged dark. The inhibition zone was pictured bright due to some amount of light passing through it. The brightness depends upon the amount of clear area due to anti-algal effects. Stronger the inhibition zone, brighter it was imaged due to

more amount of light passing through it. The images were taken at 5X magnification as beyond that it was difficult to distinguish the inhibition zone from rest of the plate area. The focus in that case would have been the single portion.

3.4.8 Dilatometry

Dilatometry was done to study the thermal expansion of various polymer blends. The specific apparatus used was Dil 2012 std, ORTON Dilatometer, S type Thermocouple. The graph was automatically plotted by the apparatus between Percentage linear change and temperature.

RESULTS & DISCUSSIONS

4.1 Anti-Algal Studies

4.1.1 Agar Disk Diffusion Method

The details of agar disk Diffusion method and associated protocol is already discussed in the experimental section. There were a total of three type of algal strains used namely Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2), Pectinodesmus sp. strain HM3 (PHM3). Each algal strain was cultured on a separate petri plate. There were a total of 6 petri plates (2 plates for each algal strain). All of the three algal strains were not only tested against various polymer blends but also with respect to different weight concentrations of PEG & SS that were used as anti-algal agents. The polymer blends were diluted in Dimethyl sulfoxide (DMSO) while PEG and SS dilutions were made in distilled water. The details of various samples that were tested for anti-algal properties are given below in table (7).

Table 7: Details of Various Samples Tested in Disk Diffusion Method

Sample Name	Sample Weight	Dilution Description
S0	2g	Pure PS in 20ml DMSO solution
Sa	2g	5% PEG/ PS blend in 20ml DMSO solution
Sb	2g	10% PEG/ PS blend in 20ml DMSO solution
Sc	2g	15% PEG/ PS blend in 20ml DMSO solution
Sd	2g	20% PEG/ PS blend in blend 20ml DMSO solution
Se	2g	10% SS/ PS blend in 20ml DMSO solution
Sf	2g	10% SS/ 10%PEG/ PS blend in 20ml DMSO solution
S1	0.05g	0.05g PEG in 15ml DMSO solution
S2	0.1g	0.1g PEG in 15ml DMSO solution

S3	0.15g	0.15g PEG in 15ml DMSO solution
S4	0.2g	0.2g PEG in 15ml DMSO solution
S5	0.1g	0.1g SS in 15ml DMSO solution
S6	0.2g	2g (0.1g PEG +0.1g SS) in 15ml DMSO solution

4.1.2 Photographic Images of Algal Cultured Petri Plates

The images of petri plates having different types of algae cultures tested against various samples are given in fig (4) and fig (5). The inhibition zones corresponding to different weight concentrations of anti-algal agents PEG and SS are imaged in fig (4). The anti-algal behavior of various polymer blends with respect to their inhibition zones are photographed in fig (5).
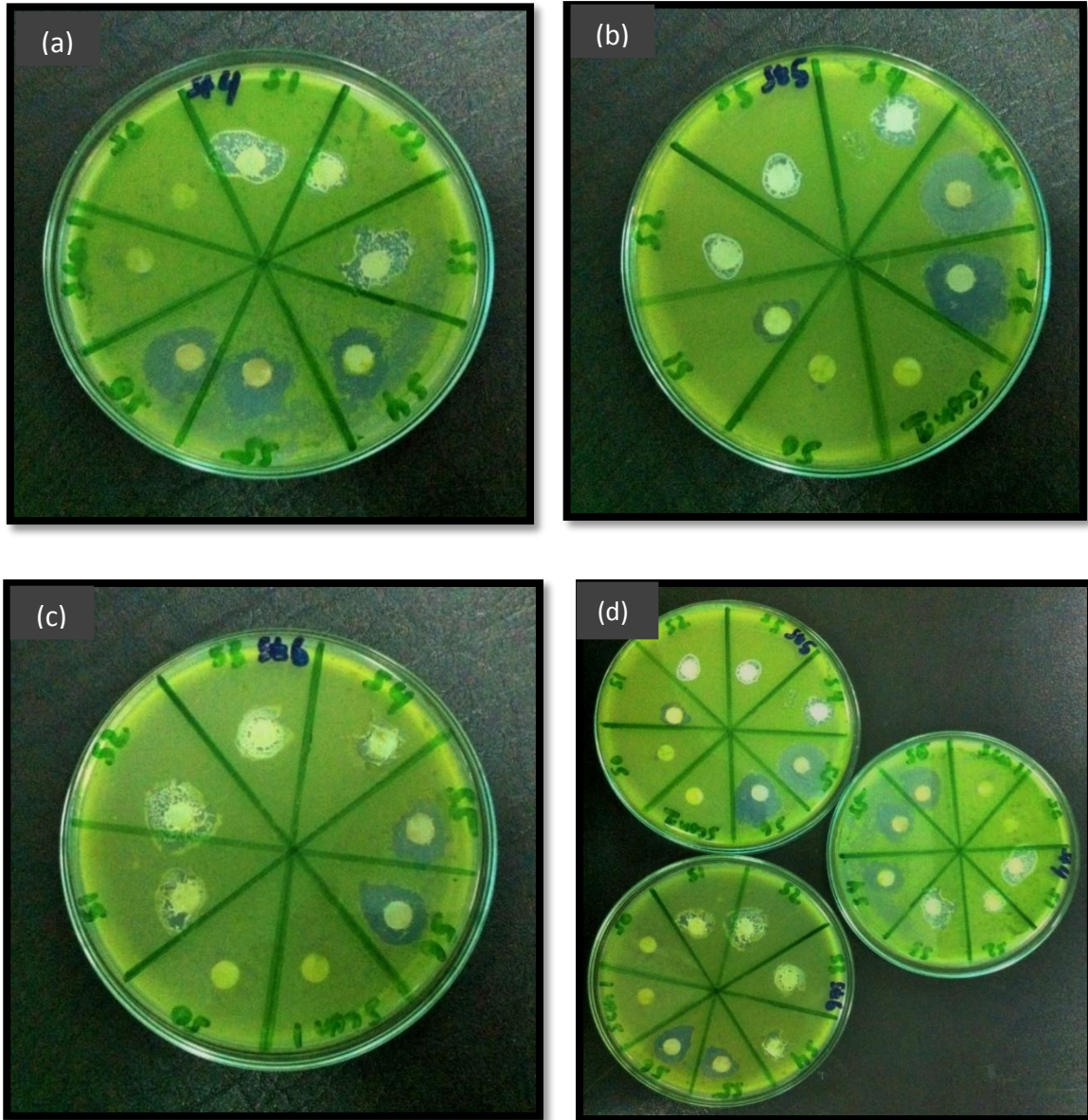
Fig 5: Inhibition zones corresponding to different Anti-Algal Concentrations against **(a)** Dictyosphaerium sp. strain HM1 (DHM1) **(b)** Dictyosphaerium sp. strain HM2 (DHM2) **(c)** Pectinodesmus sp. strain HM3 (PHM3) **(d)** Combined Image against all three Algal Strains

Fig4 (a, b, c) shows algae cultured petri plates using three different algal strains, namely Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3) respectively. Eight different types of samples including various PEG weight concentrations (S1, S2, S3, S4, S5 & S6), distilled water (Scon 1) and pure Polystyrene (S0) were deposited on the plates. The details of samples are already given in table (7). Fig (4d) is a combined image of all the three types of strains in a single view to make a comparison.

The majority of green portion in plates shows the algal growth. There are eight different portions, almost of equal area for each sample type. In each portion there is a paper disk located at almost center around which inhibition zone exists. Inhibition zone is actually the cleared area which don't allow algae to be grown. The zone of inhibition depends upon the sample type, amount of anti-algal agent and diffusion of anti-algal agent. Besides a few irregularities, a clear sequence can be seen, in accordance with the expected results which proves that PEG & SS are strong anti-algal agents.

Fig 5(a, b, c) shows cultured petri plates using the same strains but now to check the antifouling behavior of polymer blends. Fig (5d) is a combined image of the three plates in this particular case. Six different types of polymer blends (Sa, Sb, Sc, Sd, Se & Sf) including DMSO were deposited on the plates. Polystyrene is deposited at the center.

The green portion again represents algal growth. There are six different portions, almost of equal area for each sample type. Different clear zones mostly in circular shape can be seen around most of the paper disks which are inhibition zone around which inhibition of algal growth has occurred. Besides only a few irregularities, a clear sequence can be seen, in accordance with the expected results.
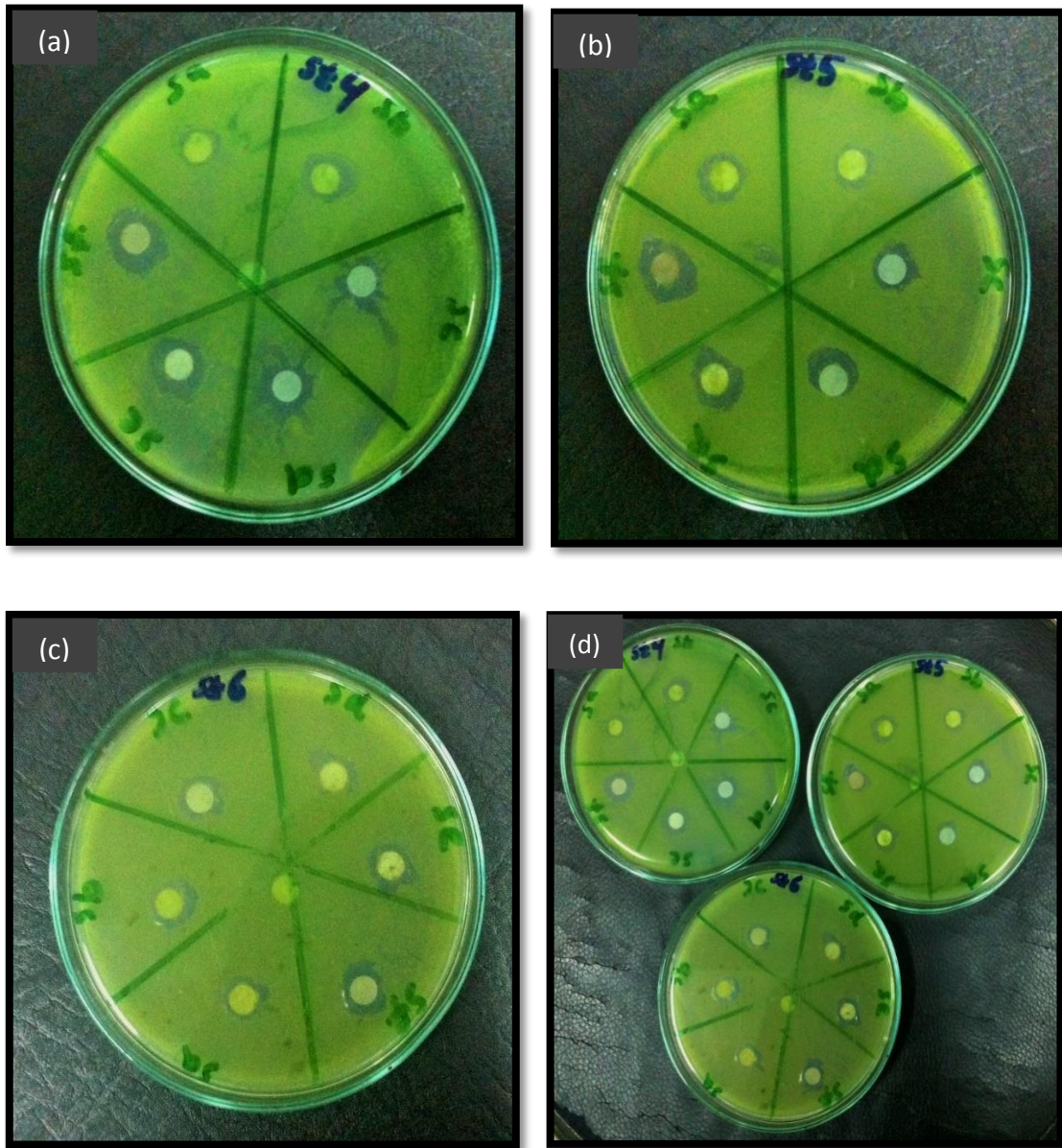
Fig 5: Inhibition zones corresponding to different Polymer blends against **(a)** Dictyosphaerium sp. strain HM1 (DHM1) **(b)** Dictyosphaerium sp. strain HM2 (DHM2) **(c)** Pectinodesmus sp. strain HM3 (PHM3) **(d)** Combined Image against all three Algal Strains

From the images discussed so far, it can be analyzed that:

I. Poly (ethylene glycol) (PEG-1000) is an anti-algal polymer.

II. Silver sulfadiazine (SS) is an anti-algal agent.

III. So PEG or SS based Polystyrene blend should also be anti-algal which is in accordance with the results seen so far.

IV. There is a direct relationship between amount of anti-algal agent and anti-fouling performance of agent or polymer blend.

V. The anti-algal character also depends upon the diffusion of agent and many other factors which can slightly modify the expected results.

All of the different types of samples have different zones of inhibition depending on the amount and diffusion of PEG and Silver sulfadiazine. As the amount of PEG is increased zone of inhibition has also increased resulting in superior anti-algal performance starting from S0 to S4 as the concentration increased from 5% to 20% by weight. There is little irregularity in case of S1 where inhibition zone seems to be higher than S2. This may be due to more amount of PEG deposited in solvent and also due to greater amount of diffusion in particular direction as it can be seen that inhibition zone is not circular enough but rather irregular. S5 shows even greater inhibition zone due to presence of a very strong anti-fouling agent SS that was used 10%. The results of S6 are best among all due to combined anti-fouling effects of PEG and SS which gave expected synergetic effect. The combined anti-algal behavior was seen in the last case where both ingredients reinforced each other.

4.1.3 Mechanism of Algal Inhibition

The paper disk wetted with respective dilution is firstly put on the petri plate instantly after algae spreading. The disk is wetted with the solvent that is non-antifouling. This solvent thus does not stay intact. Being liquid it keeps on moving in circular direction, moving radially outwards of the disk. It also carries the antifouling ingredients with it which diffuse radially outwards through the paper disk and start to act on microbial growth on the petri plate. Thus it creates inhibition zone. Ideally this inhibition zone should be

circular, but practically it is irregular depending upon the amount of anti-microbial agent and its diffusion. The ideal case is difficult to achieve practically because it is extremely difficult that same amount of antifouling agent diffuses out in all directions.

4.1.4 Measurement of Anti-algal Properties

There a variety of quantitative methods to measure Anti-fouling properties. As far as anti-algal properties are concerned the two excellent methods reported in literature are given below. Both methods measure IAD which is abbreviation of Inhibition of Algal Growth by Disk Diffusion Method. The first method deals with diameter of inhibition zone measuring IAD (a) and the second one takes in to account the area of inhibition zone calculating IAD (b). The formulas to measure both parameters are given below.

I. $IAD(a) = (d_{clear} - d_{specimen})/2$

Where IAD(a) is abbreviation of "Inhibition of algal Growth by disk Diffusion Method" using diameter of inhibition zone

d_{clear} is diameter of algal inhibition zone

$d_{specimen}$ is diameter of disk excluding algal inhibition zone

II. $IAD(b) = (A2-A1)$

Where IAD(b) stands for "Inhibition of algal Growth by disk Diffusion Method" using area of inhibition zone

A2 is area of inhibition zone

A1 is area of disk excluding algal inhibition zone

The IAD (a) and IAD (b) parameters of various samples against the different types of algal strains namely Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3) are listed in Table (8-10).

Table 8: IAD (a) and IAD (b) factors against Dictyosphaerium sp. strain HM1 (DHM1)

Sample Name	IAD(a) (mm)	IAD(b) (mm^2)
S0	0.0	0.0
S1	4.5	134.2
S2	3.8	103.0
S3	4.6	139.8
S4	5.3	169.0
S5	6.3	220.8
S6	6.8	249.0
Sa	2.5	58.9
Sb	3.0	75.4
Sc	3.1	79.7
Sd	3.3	84.2
Se	4.3	123.4
Sf	4.8	145.4
Con 1	0.0	0.0
Con 2	0.0	0.0

Table 9: IAD(a) and IAD(b) factors against Dictyosphaerium sp.strain HM2 (DHM2)

Sample Name	IAD(a) (mm)	IAD(b) (mm^2)
S0	0.0	0.0
S1	4.1	118.2
S2	4.1	118.2
S3	4.1	118.2
S4	4.4	128.8
S5	8.0	326.6
S6	8.3	343.2
Sa	2.9	71.1
Sb	2.8	66.9
Sc	3.0	75.4
Sd	3.3	84.2
Se	4.3	123.4
Sf	4.8	145.4
Con 1	0.0	0.0
Con 2	0.0	0.0

Table 10: IAD (a) and IAD (b) factors against Pectinodesmus sp. strain HM3 (PHM3)

Sample Name	IAD(a) (mm)	IAD(b) (mm^2)
S0	0	0.0
S1	4.3	123.4
S2	5.3	169.0
S3	4.3	123.4
S4	4.1	118.2
S5	5.5	181.3
S6	6.0	207.2
Sa	2.5	58.9
Sb	3.0	75.4
Sc	2.8	66.9
Sd	3.8	103.0
Se	4.8	145.4
Sf	5.3	169.0
Con 1	0.0	0.0
Con 2	0.0	0.0

4.1.5 Graphical Explanation of Anti-Algal Behavior

Fig (6) shows the anti-algal behavior of various samples against Dictyosphaerium sp. strain HM1 (DHM1). IAD (a) values are mentioned against each sample. It can be seen that S0 have no inhibition zone, therefore there IAD values are zero showing that Polystyrene is non-antifouling. Let's take in to account the anti-algal agent like PEG first. It can be seen that as the amount of PEG is increased, the IAD value has increased. This means a direct relationship between anti-algal properties and amount of anti-fouling species. This increase in values from S1 to S4 is clearly visible in graph. As the concentration of PEG is increased from 0.05 to 0. 2 g, IAD values have raised to 5.3mm from 4.5mm. There is little irregularity in S2 whose IAD value is less than S1. This is an exception which may be due to less amount of diffusion or even due to lesser amount of PEG been deposited on S2. These values further increase for S5 and S6 due to presence of a very strong anti-fouling agent Silver sulfadiazine. S5 is pure Silver sulfadiazine whose 0.1g concentration gave better results than 0.2g PEG in same experiment (compare IAD (a) values of S4 and S5). S6 produced even better results due to synergetic effect of PEG and SS which reinforced each other.

The other section of IAD values represent the antifouling behavior of PEG and SS based polymer blends. The IAD value is lowest for Sa which is in accordance with expected results because it contains minimum amount of PEG. As the amount of PEG is increased from 5% to 20% starting from Sa to Sd, IAD (a) value has increased from 2.5mm to 3.3mm. This again shows the direct relationship between the amount of anti-fouling agent and antifouling properties. The value keeps on increasing till Sf which got the maximum value and thus has maximum anti-algal behavior among various blends. Se has second highest IAD value due to presence of Silver sulfadiazine. It is important to mention that Se have half of the weight percentage of anti-algal agent in comparison with Sd but its IAD value is still higher (4.3mm in comparison with 3.3mm respectively). This is due to the presence of more active antifouling agent Silver sulfadiazine. This also shows that SS is more intense anti-algal powder.

Sf have both the agents i.e. PEG and SS in equal percentage, so it produced the most prominent anti-algal behavior. The amount of anti-algal species in Sf is same as Sd but IAD values are much higher due to reinforcement effect of PEG and SS.

	So	S1	S2	S3	S4	S5	S6	Sa	Sb	Sc	Sd	Se	Sf
IAD(a) (mm)	0.0	4.5	3.8	4.6	5.3	6.3	6.8	2.5	3.0	3.1	3.3	4.3	4.8
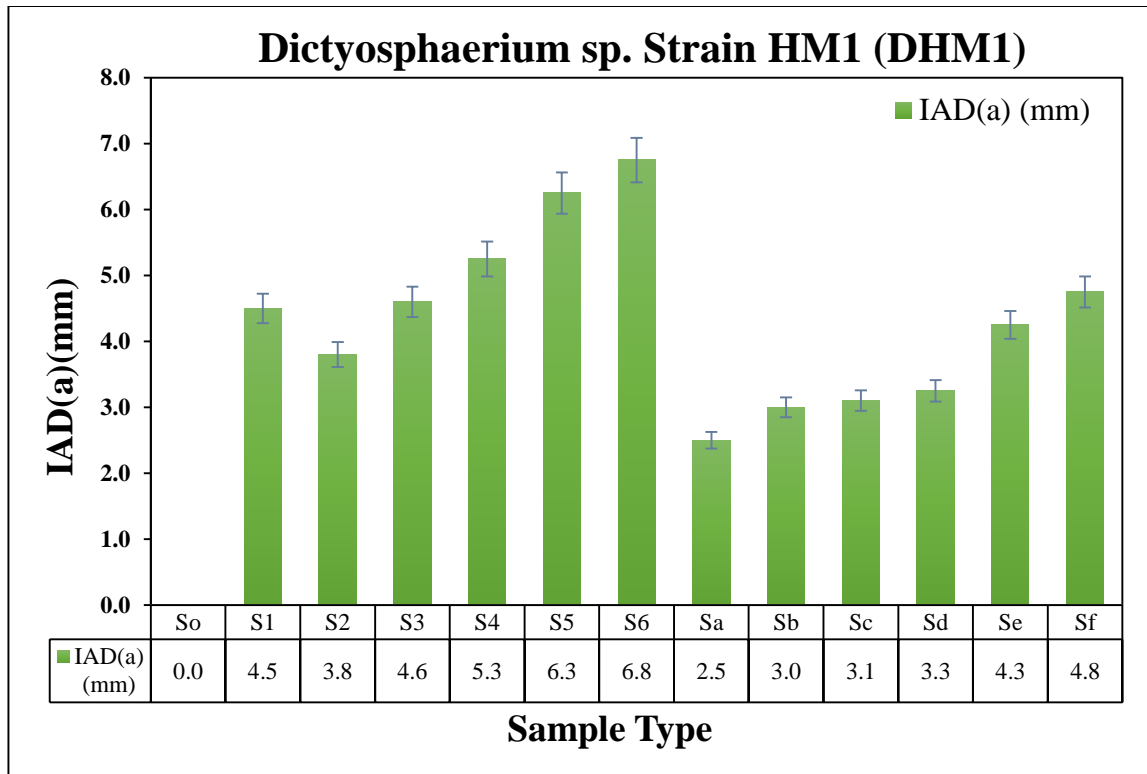
Fig 6: IAD (a) Plots of various Samples against Dictyosphaerium sp. strain HM1 (DHM1)

Fig (7) shows the IAD (a) values of various samples tested against Dictyosphaerium sp. strain HM2 (DHM2) calculated with respect to diameter of inhibition zone. It can be seen that Polystyrene did not show any anti-fouling activity, therefore there IAD values are zero showing that it is are non-antifouling. Let's discuss the anti-algal properties of PEG first. It can be seen that as the amount of PEG is increased, the IAD values either increased or remained same. There is actually a direct relationship between anti-algal behavior and amount of anti-fouling agent. The value of IAD is same i.e. 4.1mm for initial three samples i.e. S1, S2 and S3. Again there may be diffusion related issues in S2 and S3. Apparently it seems that S1 has better penetration of anti-algal agent although it has lesser amount of PEG. These same values may also be due to the fact that IAD factor takes in to account the average diameter. This average diameter is calculated taking the average of two diameters measured along x axis and y axis. It may be possible that in one direction

penetration is high, so it raises the average diameter and thus IAD value. As the concentration of PEG is increased from 0.05 to 0.2 g IAD values have raised from 4.1mm to 4.4mm due to increasing amount of PEG. The IAD values further increased in later samples S5 and S6 as clearly visible in graph. This is due to presence of a very strong anti-fouling agent Silver sulfadiazine. S5 is pure SS whose 0.1g concentration gave better results than 0.2g PEG in same experiment (compare IAD values of S4 and S5). S6 produced even better results due to synergetic effect of PEG and SS.

The other section of IAD values present the antifouling behavior of PEG and Silver sulfadiazine based polymer blends. The IAD value is lowest for Sb. This is little irregularity in the results. The lowest value should be of Sa but such ambiguities may exist due to diffusion problems as discussed earlier. As the amount of PEG is increased from 5% to 20% starting from Sa to Sd, IAD value has increased from 2.9mm to 3.3mm. This again shows the direct relationship between the amount of anti-fouling agent and its anti-fouling properties. The value keeps on increasing till Sf which got the maximum value and thus the maximum anti-algal behavior among various blends. Se has second highest value due to presence of Silver sulfadiazine. It is important to mention that Se have half of the weight percentage of anti-algal agent in comparison with Sd but its IAD value is still higher (4.3mm in comparison with 3.3mm respectively). This is due to the presence of more active antifouling agent.

Sf have both the agents i.e. PEG and SS in equal percentage, so it produced the most prominent anti-algal behavior. The amount of anti-algal species in Sf is same as Sd but IAD values are much higher due to reinforcement effect of PEG and SS.

Dictyosphaerium sp. Strain HM2 (DHM2)

|	So	S1	S2	S3	S4	S5	S6	Sa	Sb	Sc	Sd	Se	Sf
IAD(a) (mm)	0.0	4.1	4.1	4.1	4.4	8.0	8.3	2.9	2.8	3.0	3.3	4.3	4.8
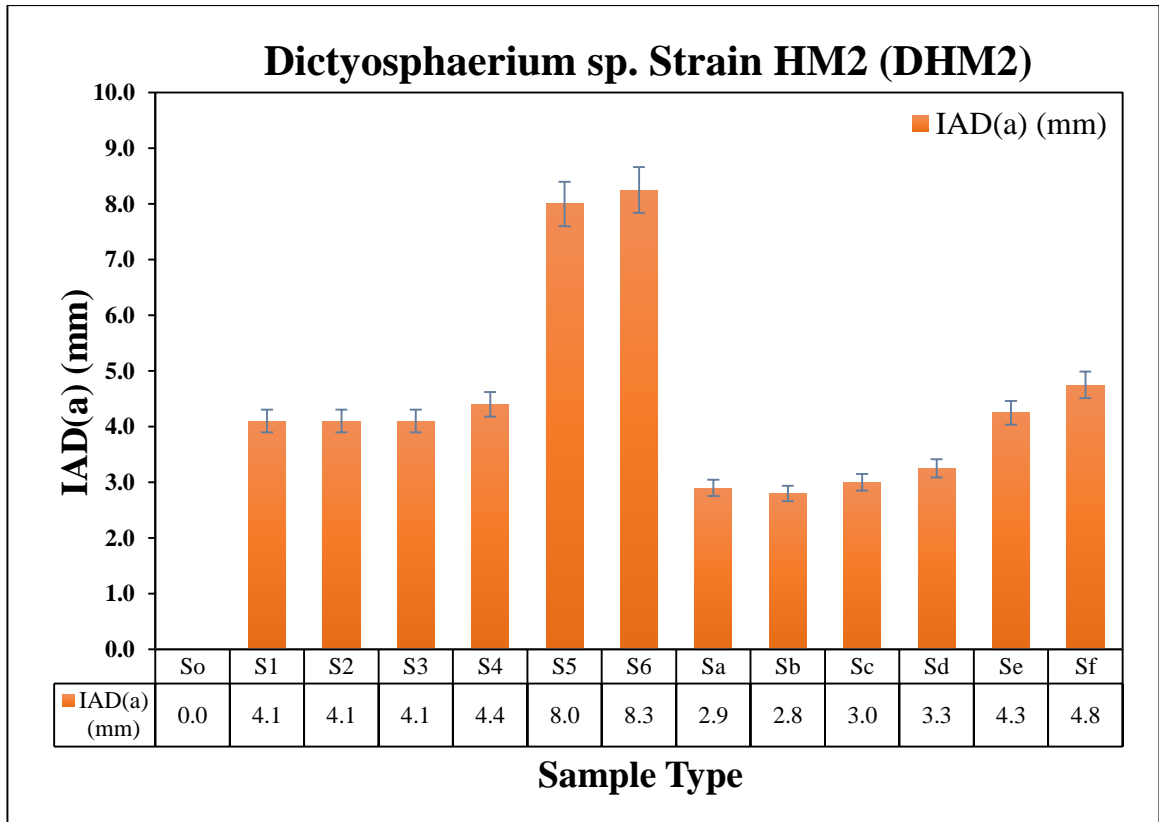
Fig 7: IAD (a) Plots of various Samples against Dictyosphaerium sp. strain HM2 (DHM2)

Fig (8) shows the IAD (a) values of various samples against Pectinodesmus sp. strain HM3 (PHM3) 6 calculated with respect to inhibition zone diameter. The IAD (a) values are again zero for Polystyrene showing that PS is non-antifouling. The anti-algal properties of PEG are discussed first. There is a bit of irregular behavior seen in present case. For example the IAD value increased initially as the concentration of PEG is increased from 5% to 10% in S1 and S2. The value of S3 is same as S1. The value in case of S4 is even less than S1, S2 and S3. Despite some unexpected results there is certain inhibition zone in all cases, indicating the inhibition of algal growth. So there are scattered values in present case. The IAD values are increased in S5 and S6 .These results are in accordance with the previous results. The IAD factor is raised from 4.1mm to 6mm showing the noticeable effect of Silver sulfadiazine. This is due to presence of a very strong anti-fouling agent Silver sulfadiazine. S5 is pure SS whose 0.1g concentration gave better results than 0.2g PEG in same experiment (compare IAD values of S4 and S5). S6 produced even better results due to synergetic effect of PEG and SS which reinforced each other.

The other section of IAD (a) values represent the antifouling behavior of PEG and SS based polymer blends. The IAD value is lowest for Sa. The value increases from 2.5mm to 3mm as the concentration of PEG is increased from 5% to 10%. The IAD value again drops to 2.8mm as the concentration is further increased to 15% in Sc. The irregularity in the results can be due to a number of factors. The effectiveness of anti-algal agent depends on how much the agent is attached to the paper disk and how much diffused across radially. As the amount of PEG is increased from 5% to 20% starting from Sa to Sd, IAD value has increased from 2.5mm to 3.8mm. This again shows the direct relationship between the amount of anti-fouling agent and antifouling properties. The value keeps on increasing till Sf which got the maximum value and thus maximum anti-algal behavior among various blends. Se has second highest IAD value due to presence of Silver sulfadiazine. It is important to mention that Se have half of the weight percentage of anti-algal agent in comparison with Sd but its IAD value is much higher i.e 3.8mm for Sd in comparison with Se whose IAD value is 4.8mm respectively. This is due to the presence of stronger antifouling agent Silver sulfadiazine.

Sf have both the agents i.e. PEG and silver SS in equal percentage, so it produced the most prominent anti-algal behavior. The amount of anti-algal agent in Sd is same as Sf but IAD values are much higher due to reinforcement effect of PEG and Silver sulfadiazine in later case.
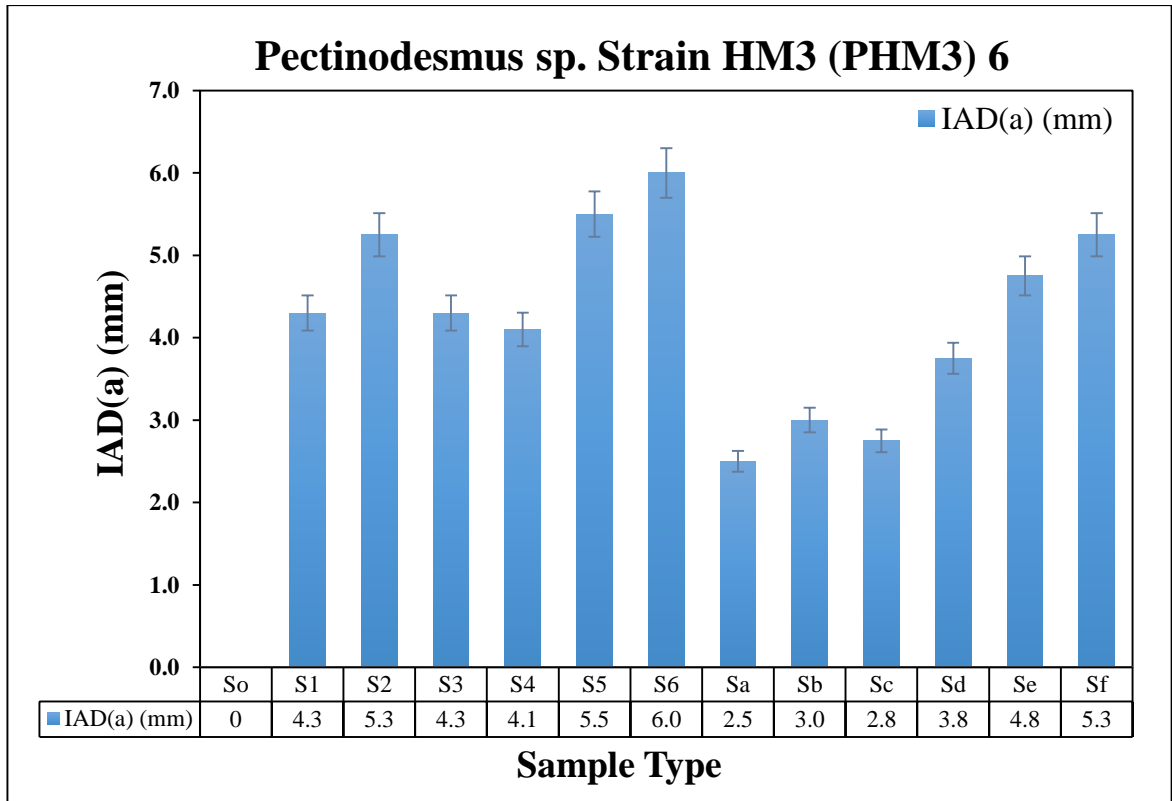
Fig 8: IAD (a) Plots of various Samples against Pectinodesmus sp. strain HM3 (PHM3)

Fig (9) shows the combined results of three different algal types in a same graph. The regular trend is that as the amount of anti-algal agent is increased, IAD value increases, indicating increase in anti-fouling character. This trend can be seen both in polymer blends and anti-algal agents alone. The more pronounced effect of SS in comparison with PEG can also be observed. Present figure shows that anti-algal agents and their polymer blends are effective against every algal strain. A general conclusion cannot be drawn about which algal strain is more prone to the anti-fouling agents. Some samples are damaging to Dictyosphaerium sp. strain HM1 (DHM1), some are more prone to Dictyosphaerium sp. strain HM2 (DHM2), while remaining inhibit Pectinodesmus sp. strain HM3 (PHM3) effectively.
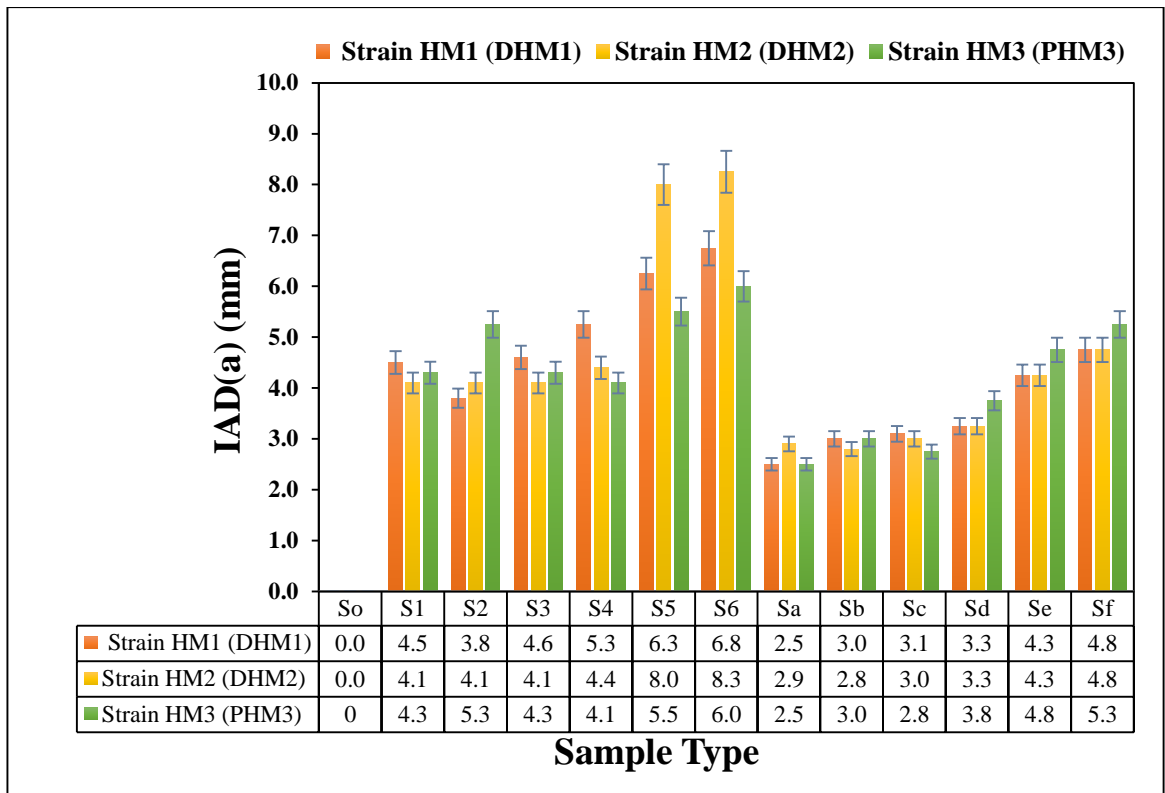
Fig 9: Combined IAD (a) Plots of various Samples against all three Algal Strains

Fig (10-11) plot the IAD (b) values of different samples tested against Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3). IAD (b) is inhibition of algal growth by disk diffusion method which uses area for its calculation. IAD (b) is a better measurement because it takes in to account the area of inhibition zone instead of diameter, which gives more prominent numbers. The overall trend in all upcoming graphs is same as IAD (a). As the amount of anti-algal agent is increases, IAD (b) has also increased in most of the cases. There is a direct relationship between IAD (b) and concentration of antifouling agent in most of the cases. The trends of IAD (a) graphs are same as IAD (b) graphs in respective algal types. The regular trend is that as the amount of anti-algal species is increased, IAD value increases, indicating increase in anti-fouling character. This trend can be seen both in polymer blends and anti-algal agents alone. The more pronounced effect of SS in comparison with PEG can also be observed.

Figure (13) shows that anti-algal agents and their polymer blends are effective against every algal strain as far as IAD (b) is concerned. A general conclusion cannot be drawn about the magnitude of damage done by particular algal strain against certain agent, blend or concentration. Some samples damage Dictyosphaerium sp. strain HM1 (DHM1) effectively, some inhibit Dictyosphaerium sp. strain HM2 (DHM2) growth noticeably, while other samples inhibit Pectinodesmus sp. strain HM3 (PHM3) effectively. The only difference between first four set of graphs and last 4 set of graphs is that one takes on diameter while other uses area to calculate the inhibition of algal growth. The trends of respective graphs are completely same, only difference is in numbers. IAD (a) gives small values while IAD (b) gives little big numbers. The first one considers diameter while second one considers area of inhibition zone, which may be more effective.

Dictyosphaerium sp. Strain HM1 (DHM1)

	So	S1	S2	S3	S4	S5	S6	Sa	Sb	Sc	Sd	Se	Sf
IAD(b) (mm2)	0.0	134.2	103.0	139.8	169.0	220.8	249.0	58.9	75.4	79.7	84.2	123.4	145.4

Sample Type

Dictyosphaerium sp. Strain HM2 (DHM2)

	So	S1	S2	S3	S4	S5	S6	Sa	Sb	Sc	Sd	Se	Sf
IAD(b) (mm2)	0.0	118.2	118.2	118.2	128.8	326.6	343.2	71.1	66.9	75.4	84.2	123.4	145.4

Sample Type
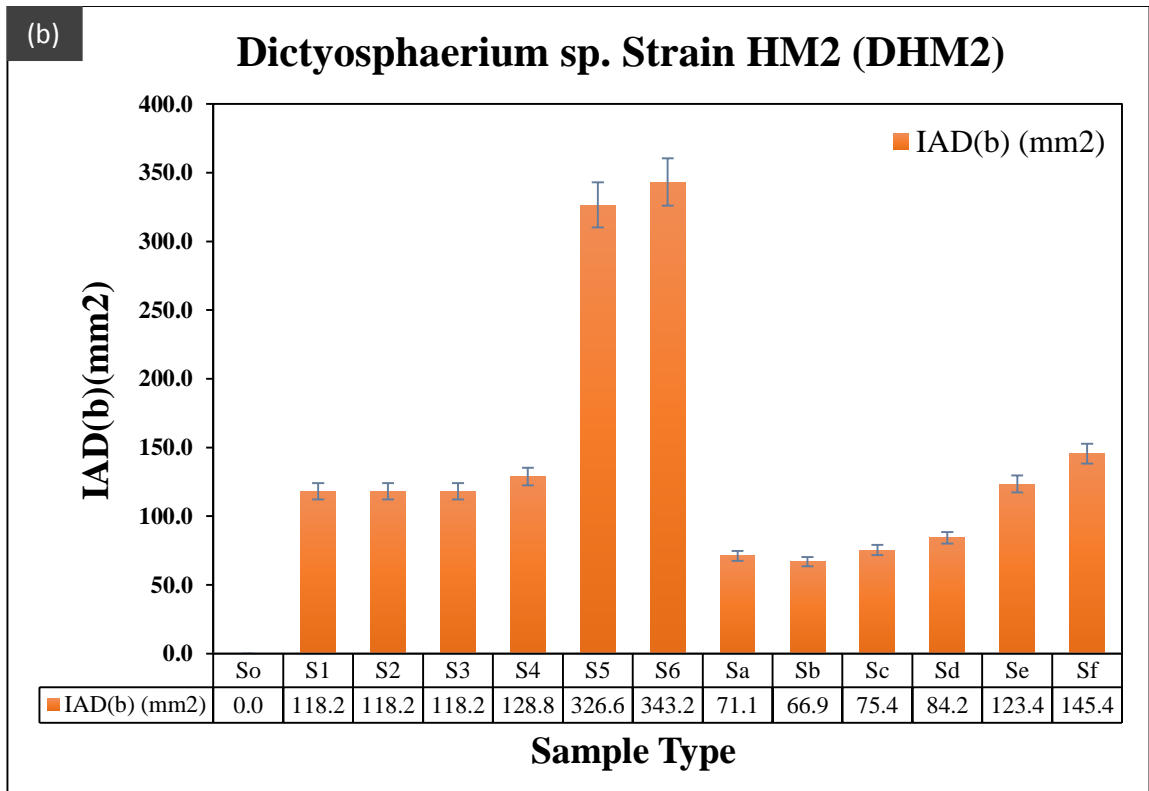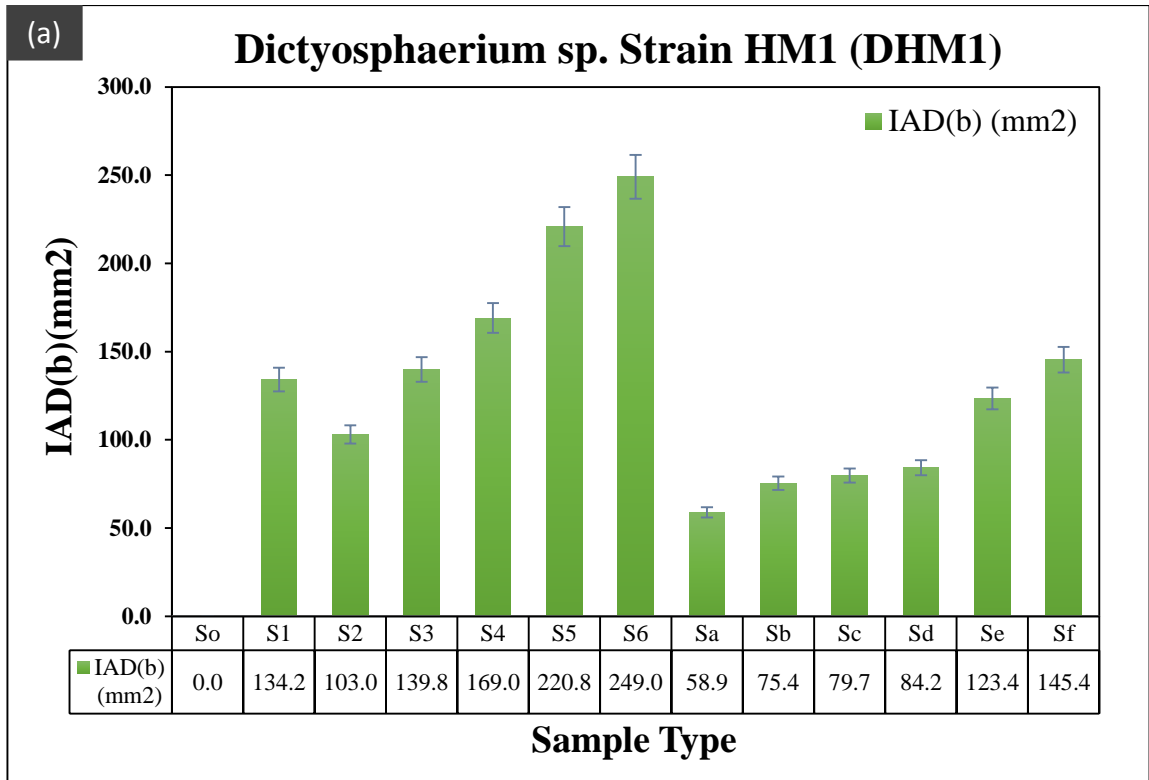
Fig 10: IAD (b) Plots of various Samples against **(a)** Dictyosphaerium sp. strain HM1 (DHM1) **(b)** Dictyosphaerium sp. strain HM2 (DHM2)

(c) Pectinodesmus sp. Strain HM3 (PHM3)

IAD(b) (mm2)	So	S1	S2	S3	S4	S5	S6	Sa	Sb	Sc	Sd	Se	Sf
IAD(b) (mm2)	0.0	123.4	169.0	123.4	118.2	181.3	207.2	58.9	75.4	66.9	103.0	145.4	169.0

(d)

	So	S1	S2	S3	S4	S5	S6	Sa	Sb	Sc	Sd	Se	Sf
Strain HM1 (DHM1)	0.0	134.2	103.0	139.8	169.0	220.8	249.0	58.9	75.4	79.7	84.2	123.4	145.4
Strain HM2 (DHM2)	0.0	118.2	118.2	118.2	128.8	326.6	343.2	71.1	66.9	75.4	84.2	123.4	145.4
Strain HM3 (PHM3)	0.0	123.4	169.0	123.4	118.2	181.3	207.2	58.9	75.4	66.9	103.0	145.4	169.0
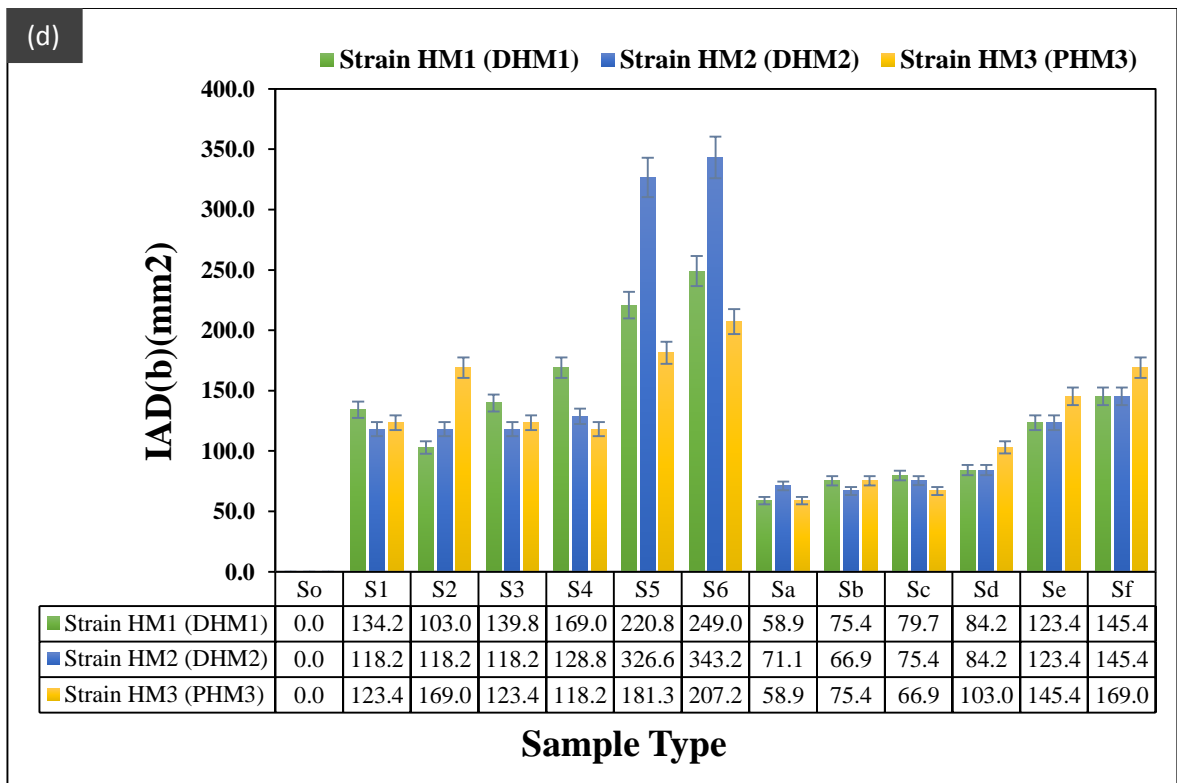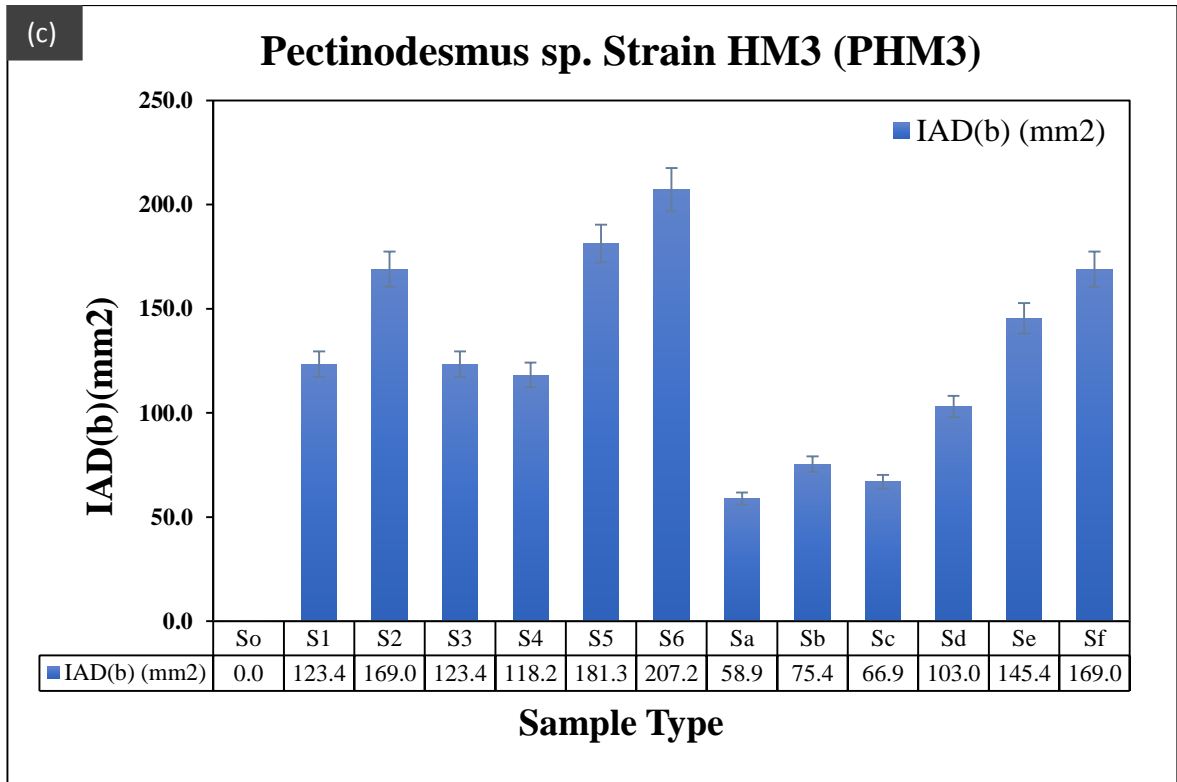
Fig 11: IAD (b) Plots of various Samples against **(a)** Pectinodesmus sp. strain HM3 (PHM3) **(b)** Combined Plot against all Algal Strain

4.2 Mechanical Testing

The complete protocol about how mechanical testing was done is already discussed in the experimental section. This section would describe the mechanical properties calculated from the respective experiment. There were mainly three properties calculated.
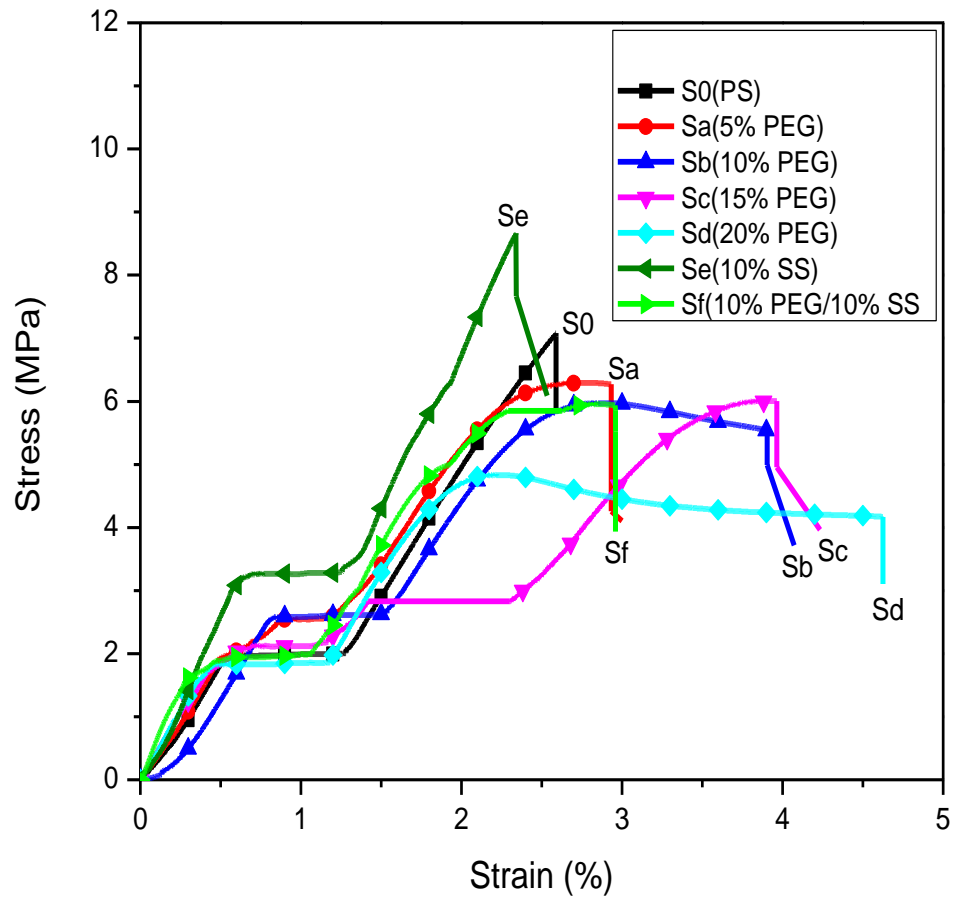
- ✓ Tensile Strength
- ✓ Elastic Modulus
- ✓ Percentage Elongation at Break

Fig (12) shows the Tensile test of various samples graphed in the same figure and scale. Basically the graphs were drawn between Percentage strain on horizontal axis and Stress in MPa on vertical axis. The experiment was done for seven different types of samples whose details are given in the graph i.e. there is varying percentage of anti-algal agents like PEG and SS in each sample.

Remember that Universal Testing can also perform a number of other tests, most prominent been compression Test and Bend Test, but we focused mainly on tensile properties of our samples. The tensile test alone can also calculate a number of important mechanical factors but we focused the experiments to calculate mainly Tensile Strength, Percent elongation at break or strain Percentage and Elastic Modulus.

The tensile strength of each sample was obtained straight away from the respective curve. It was actually the highest point in the peak where stress was maximum. Similarly elongation at break was actually the lowest point in the peak where stress was minimum or elongation as maximum. Elastic modulus was calculated from the elastic portion i.e. straight line region of the curve where slope was constant or nearly constant. Calculating modulus may be little tricky in case of polymers because it may be sometimes difficult to find out the limit of elastic region or straight line portion. Roughly seeing the graph we can find out that Tensile strength was not more than 9MPa for any sample and percentage strain was between 2.5 and 4.

Fig 12: Tensile Stress- Strain curve of various Polymer Samples

Table (11) shows Elongation at Break, Tensile strength and elastic Modulus of various blends calculated from graphs according to the method discussed in previous paragraph.

Table 11: Mechanical Properties calculated from Stress-strain Curve

| Sample Name	Tensile Strength (MPa)	% Strain	Elastic Modulus (MPa)
S0(Pure PS)	7.04	2.57	4.32
Sa(5%PEG)	6.31	3.00	4.22
Sb(10%PEG)	5.98	4.06	4.11
Sc(15%PEG)	5.95	4.24	4.21
Sd(20%PEG)	4.88	4.62	4.53
Se(10%SS)	8.58	2.53	5.97
Sf(10%PEG&10%SS	5.98	2.96	5.89

4.2.1 Tensile Strength

Fig (13) shows variation in tensile strength of various samples graphically. As the amount of PEG is increased, there is a decrease in tensile strength. The tensile stress was 7.04MPa for pure PS which dropped to 4.88 MPa for Sd where amount of PEG was 20%. So there is an inverse relation between the amount of PEG and tensile strength in PEG/PS blends. The results are in accordance with the expected calculations because PEG is a soft, flexible and ductile polymer in comparison with PS which is rigid and brittle.

The tensile strength values increase as Silver sulfadiazine is added in the blend. The blend with 10 % SS got the maximum strength 8.58 MPa. The reason is that SS itself have high tensile strength and low ductility which reinforces Polystyrene and increases the value from 7.04 to 8.58MPa due to synergetic effect. The value then decreases to 5.98MPa in Sf as the percentage of SS is decreased from 20% to 10% and adding 10% PEG. Now there are two types of ingredients in Sf i.e. PEG and SS both playing their own role. PEG tends to decrease tensile strength of blend, the reason explained earlier being softness, flexibility and lower tensile strength of PEG. SS is brittle and strong adding value to the tensile strength of polymer blend. These two components played their role and their synergetic effect managed to make the strength 5.98MPa. The value could have been much higher if there was no PEG content removing plasticity from the blend. Contrary to this, the value

could have been much lower, if there was no SS as there would be less rigidity and increased plasticity & flexibility in that case.

All of the samples during tensile loading were undergone through elastic region, followed by yielding and necking in plastic region. This can not only be proved from various curves but also from geometry of broken samples which showed slightly cup and cone behavior.

	S0	Sa	Sb	Sc	Sd	Se	Sf
Tensile Strength (Mpa)	7.04	6.31	5.98	5.95	4.88	8.58	5.98

Sample Type
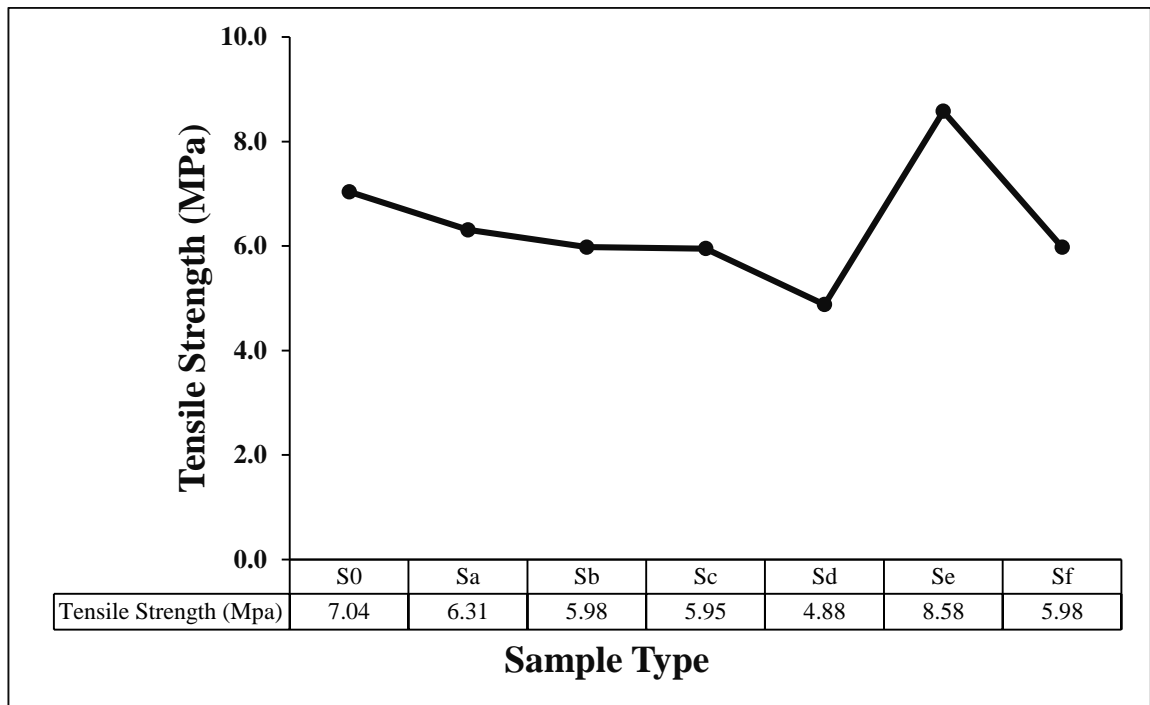
Fig 13: Variation in Tensile Strength of Polymer blends w.r.t different Compositions

4.2.2 Percentage Strain

Fig (14) explains Percentage Elongation at Break of various samples. The percentage elongation initially increased from 2.57 to 4.62 as the amount of PEG was increased from 5% to 20% starting from S0 to Sd. The enhanced elongation depicts increase in ductility and toughness which can be observed from respective plots as depicted in fig (12). This is due to increasing amount of PEG which is highly flexible, soft and ductile. This amount of PEG introduces plasticity in the blend, leading to decrease in tensile strength. So there is a direct relationship between amount of PEG and ductility & Toughness in the blend.

As 10% Silver sulfadiazine is added in to the blend, the elongation value suddenly drops to 2.53 in Se. This is due to lesser plasticity and ductility of both components which have higher degree of brittleness in comparison with PEG. In se if some amount of PEG is added i.e. (10% PEG & 10% SS in Sf) the percentage strain value increases minimally to 2.96. The rigidity and brittleness of blend is little decreased by PEG, adding flexibility and ductility to the blend.


	S0	Sa	Sb	Sc	Sd	Se	Sf
Elongation (%)	2.57	3	4.06	4.24	4.62	2.53	2.96

Sample Type
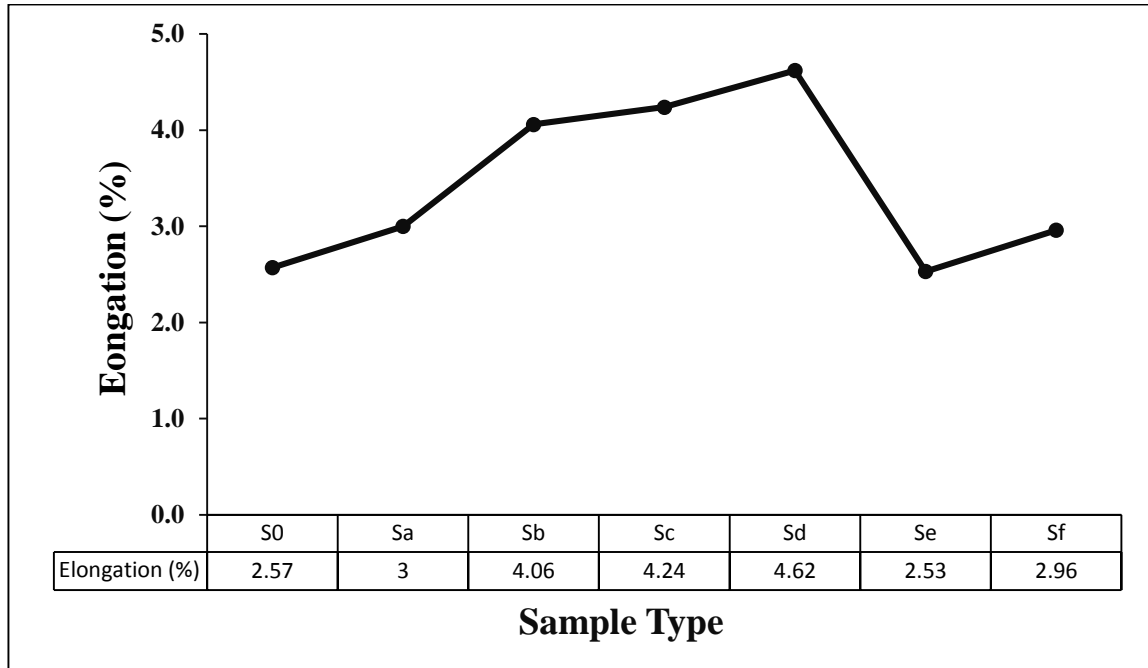
Fig 14: Variation in Percentage Elongation of Polymer blends w.r.t different Compositions

4.2.3 Elastic Modulus

Fig (15) graphically shows the trends in Elastic Modulus with respect to different samples. The results of Elastic Modulus are little unexpected and random. The modulus was 4.32 MPa for pure Polystyrene. The modulus value initially decreased to 4.22 MPa as the amount of PEG was increased to 5% in Sa. The value now decreases to 4.11MPa as the amount of PEG was increased to 10% in Sb. This decrease in modulus is due to lesser stiffness and rigidity of PEG which cannot bear much stress or load. The modulus of PEG is low itself due to higher flexibility which adds on its effect as the amount of PEG is increased. With the further increase in amount of PEG in Sc and Sd, the results are little

ambiguous. The modulus value initially increases from 4.21MPa to 4.53MPa in Sc and Sd with respective amounts of 15% and 20% PEG by weight. This irregular trend is difficult to describe. It may be due to non-homogeneous distribution of filler. The difference any ways is not too obvious.

The modulus value jumps up to 5.97 MPa in Se as Silver sulfadiazine was added in blend. This high modulus value is due to SS which is highly rigid and stiff. It can bear more amount of load since both components of blend are stiff, so their combined effect can be observed in Se. The modulus value in Sf is slightly less than Se but it's still very high than all other samples. These two last samples are enough to understand the effect of SS in polymer blend. The modulus of Sf is slightly lesser because it has equal amount of PEG just like SS. PEG being flexible and lesser stiff lowers the modulus of blend to some extent. The value is slightly lowered from 5.97MPa to 5.89MPa.


	S0	Sa	Sb	Sc	Sd	Se	Sf
Modulus (MPa)	4.32	4.22	4.11	4.21	4.53	5.97	5.89
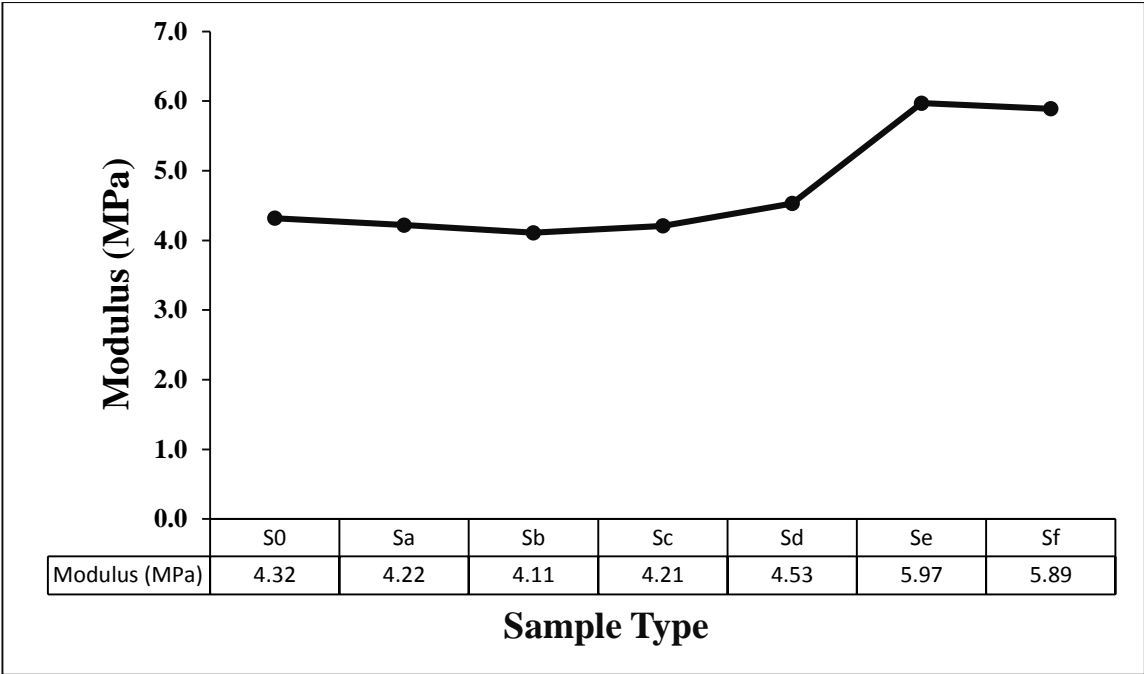
Fig 15: Variation in Elastic Modulus of Polymer blends w.r.t different Compositions

4.3 Optical Microscopy

Optical Microscopy was carried out to differentiate the inhibition zone from rest of the area. Microscopy was done for each sample portion of the petri plate. It is important to mention that instead of reflection mode, the absorption mode was used. The imaging was done across the boundary line separating inhibition zone from algal growth region. The area where algae is killed i.e. inhibition zone, there would be less absorption and maximum amount of light will pass through. That portion as a result would appear bright. The portion having algal growth would appear dark because it will not allow light to transmit through and maximum amount of light will be absorbed. The area would look dark in that case. Fig (16) shows the optical micrographs of various samples tested against algal strain Dictyosphaerium sp. strain HM1 (DHM1). All of the samples Sa, Sb, Sc, Sd, Se and Sf were imaged at 5X magnification. The idea is to see the contrast between the inhibition zone and algal growth portion within same image, so low magnification was suitable.

The clear contrast can be seen in every image. The area containing inhibition zone looks bright while remaining portion is black. The stronger the inhibition zone, the brighter it is imaged. The width of bright portion can also indirectly tell us about the width of inhibition zone but on such a small scale we cannot rely on this principle. There are different patterns in every image. These different portions are microstructures of underlying transparent agarose. Beneath algal layer, there is agar layer. When algae is cleared from certain zone it leaves away the microstructure beneath which is viewed bright actually. We are not primarily interested in microstructure of agar layer. We are more interested in brightness of inhibition zone which can indirectly tell us about the effectiveness of anti-algal agent. The images explain that Polystyrene based blends are quite effective against Dictyosphaerium sp. strain HM1 (DHM1).
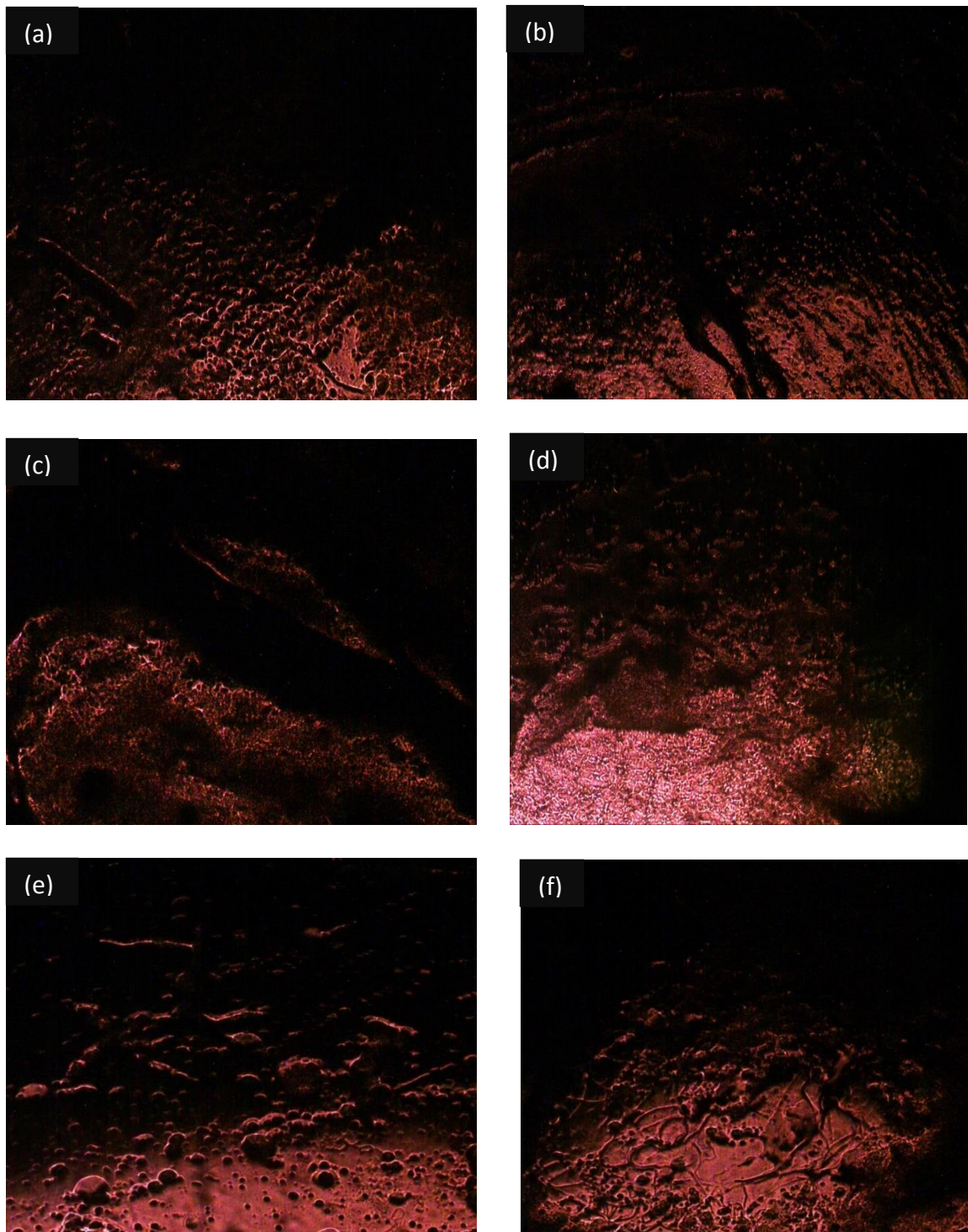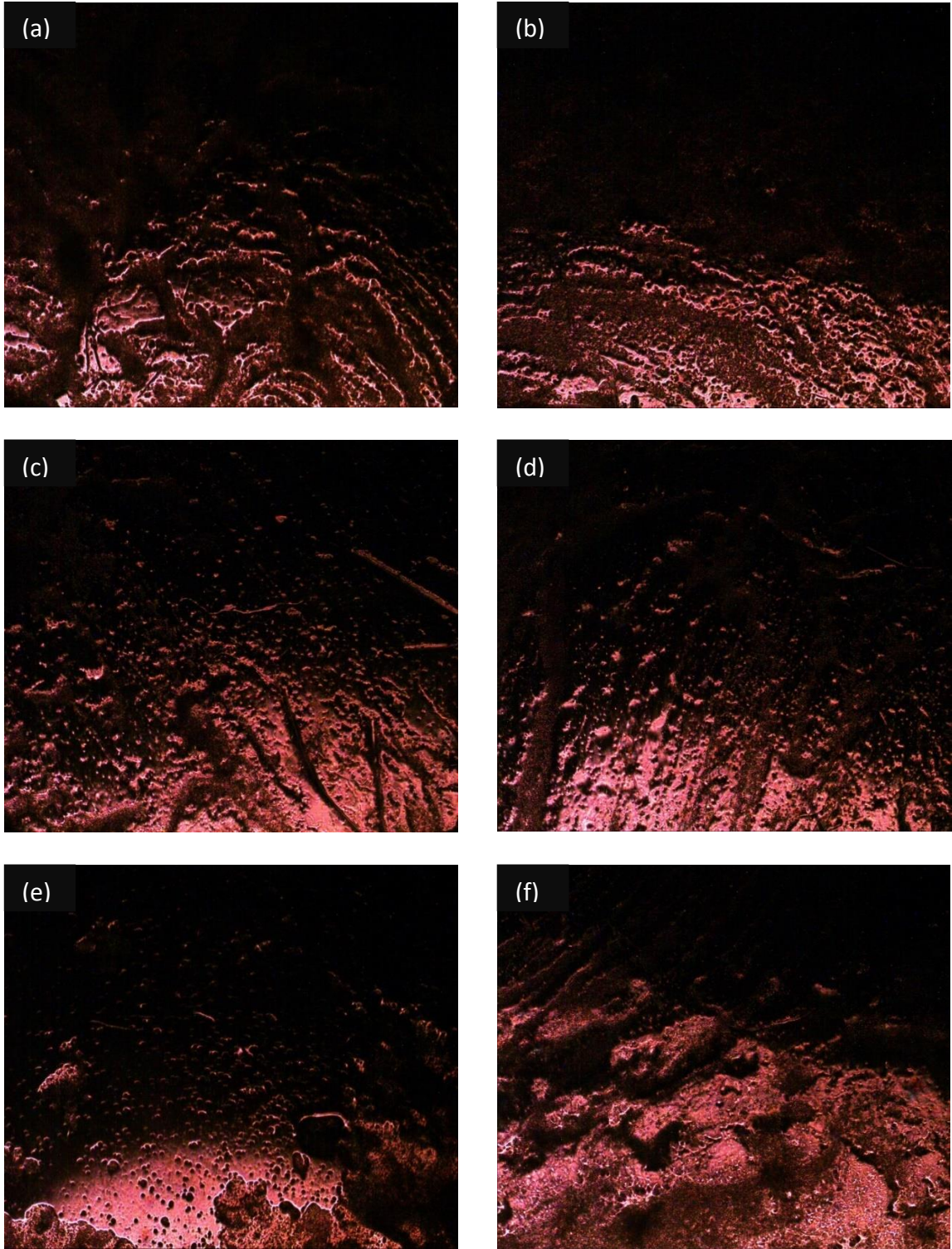
Fig 16: Optical Micrographs of **(a)**5%PEG **(b)**10%PEG **(c)**15%PEG **(d)**20%PEG **(e)**10%SS **(f)**10%PEG & 10%SS Blends Tested against Dictyosphaerium sp. strain HM1 (DHM1)

Fig (17) shows the optical micrographs of various samples tested against algal strain Dictyosphaerium sp. strain HM2 (DHM2). All of the samples Sa, Sb, Sc, Sd, Se and Sf were imaged at 5X magnification. The contrast can clearly be seen in every sample. The inhibition zone views bright while remaining portion looks dark. The brightness indicates strength of anti-algal agent. The width of bright portion can indirectly tell us about the width of inhibition zone but on such a small scale we cannot rely on this principle because whole of inhibition zone cannot be viewed by microscope, no matter how less the magnification is. There are various patterns which can be viewed in every sample. These different patterns in every sample again indicate the microstructures of underlying transparent agarose. There are smaller darker regions within the bright portion also. These indicate some growth of algae besides presence of anti-algal agent. The images clearly depict the effectiveness of Polystyrene based blends against Dictyosphaerium sp. strain HM2 (DHM2).

Fig (18) shows the optical micrographs of various samples tested against algal strain Pectinodesmus sp. strain HM3 (PHM3). The images are same as the previous samples explained. Micrographs explain the effectiveness of polymer blends against Pectinodesmus sp. strain HM3 (PHM3). All of the sample blends are effective but to the varying degree depending upon the composition of the sample. The bright portion in samples explain the transmittance of light from the inhibition zone due to absence of algae there. Rest of the petri plate appear black due to absorption of micro algae. The intensity of brightness indirectly indicates the strength of anti-fouling blend and about how effectively it worked. As discussed earlier that ee are nothing to do with the various microstructures of agar layer underneath algal layer. The brightness of various portions of petri plate is quite enough to indicate the anti-fouling effect of various polymer blends.

Fig (17): Optical Micrographs of **(a)**5%PEG **(b)**10%PEG **(c)**15%PEG (d)20%PEG **(e)** 10%SS **(f)**10%PEG & 10%SS Blend Tested against Dictyosphaerium sp. strain HM2 (DHM2)
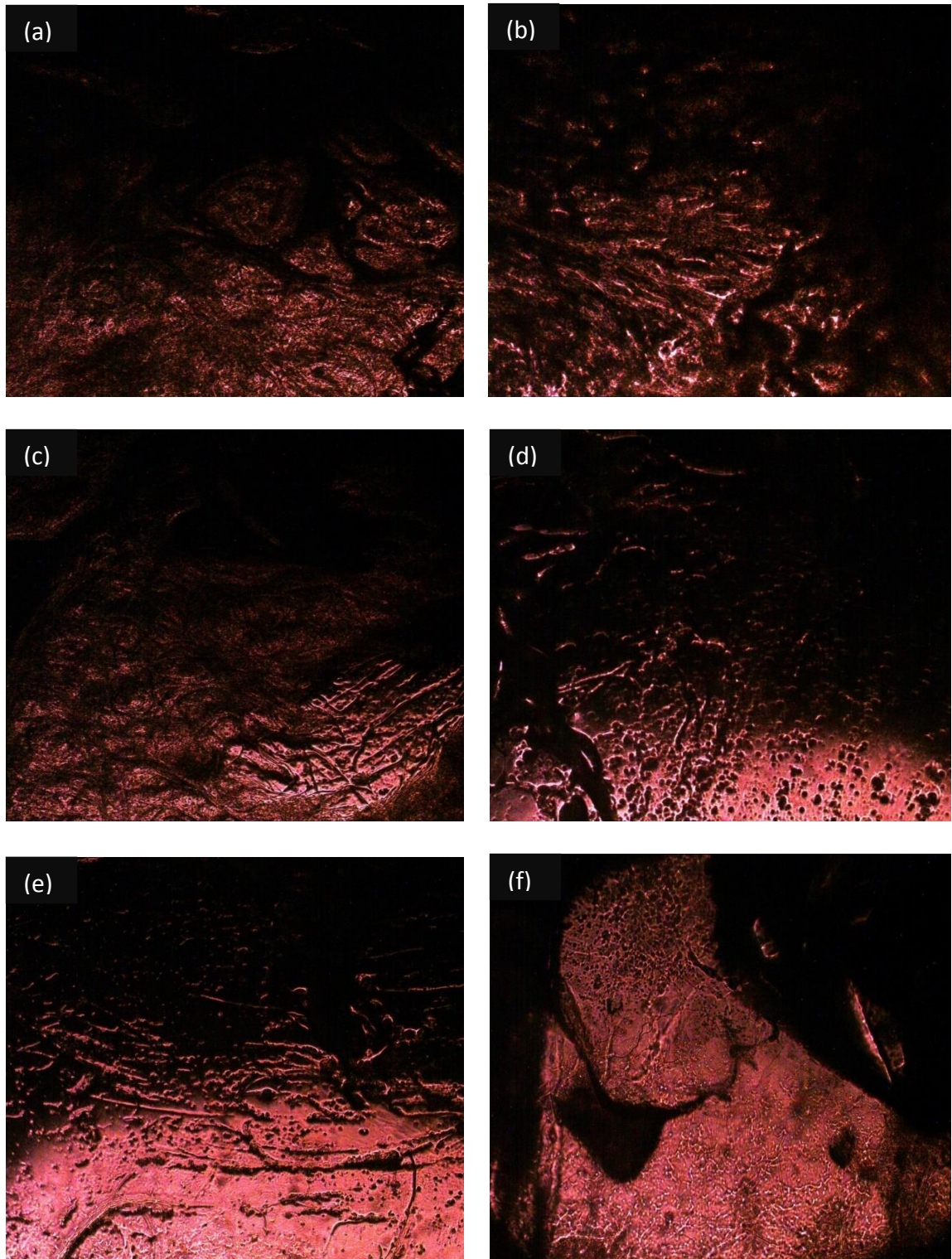
Fig (18): Optical Micrographs of **(a)**5%PEG **(b)**10%PEG **(c)**15%PEG **(d)**20%PEG **(e)** 10%SS **(f)**10%PEG & 10%SS Blend Tested against Pectinodesmus sp. strain HM3 (PHM3)

4.4 Thermal Gravimetric Analysis

TGA was done to study the percentage weight loss of polymer blends against temperature change. This can indirectly tell us about thermal stability and thermal decomposition of various samples. Fig (19) plots the TGA thermographs of various samples S0, Sa, Sb, Sc, Sd, Se and Sf respectively.
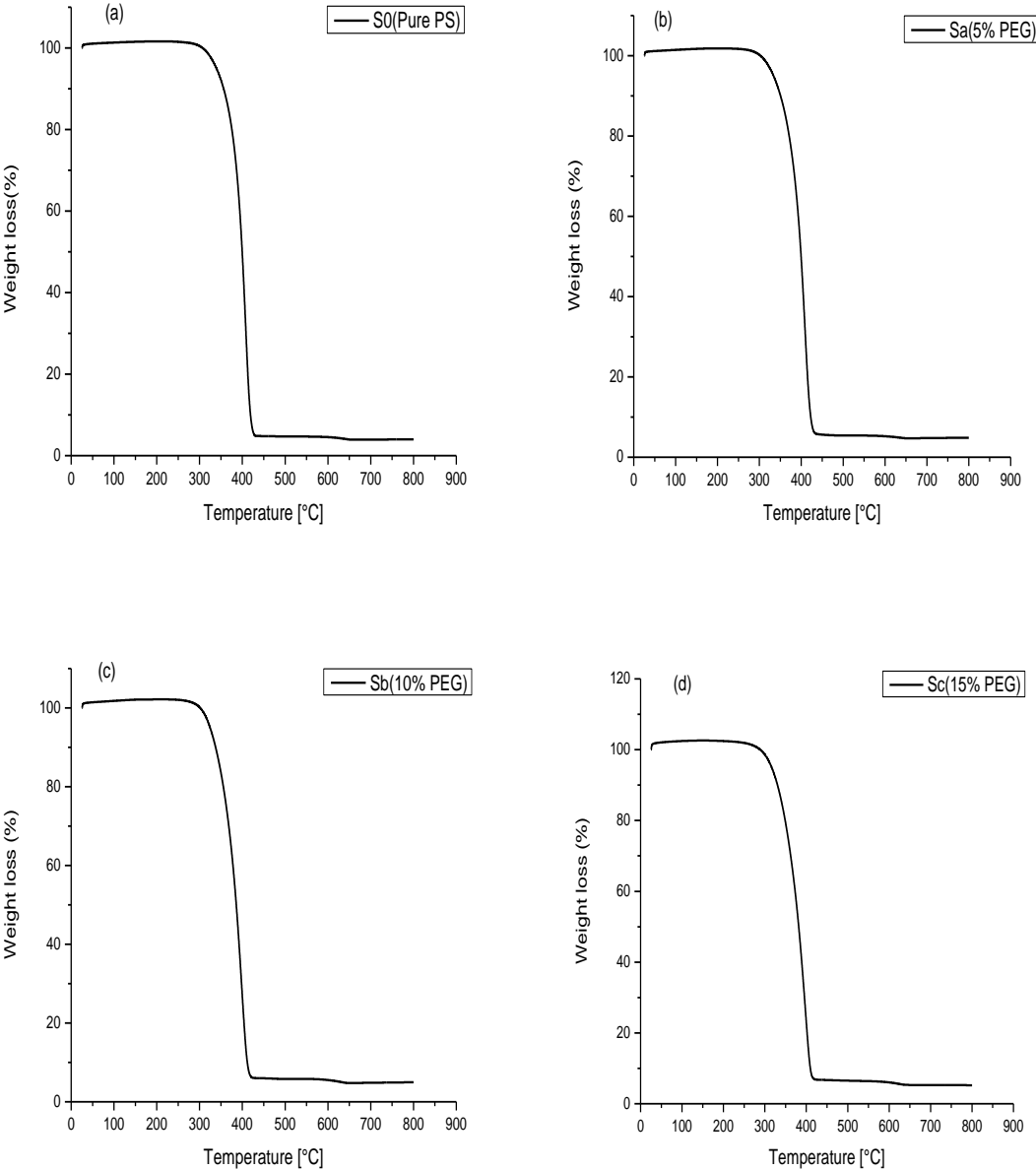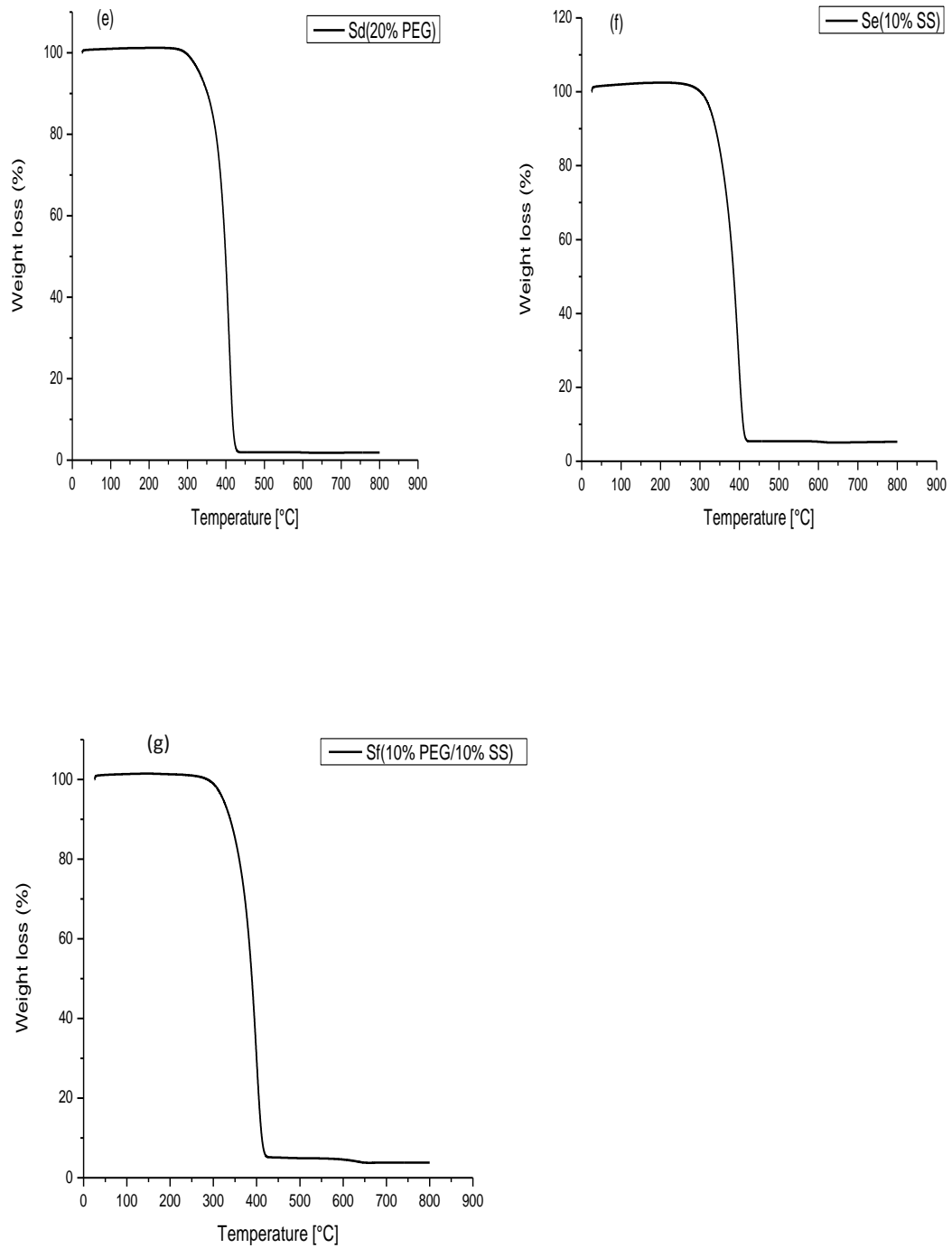
Fig 19: TGA Thermographs of **(a)** PS **(b)** 5%PEG **(c)** 10%PEG **(d)** 15%PEG **(e)** 20%PEG **(f)** 10%SS 10%PEG & 10%SS Blends

4.4.1 Combined TGA Plot

Fig (20) views the combined TGA plot of all the samples in a same frame to make out the comparison. The basic trend in all of the graphs was almost same. Figures depict that decomposition of polymer blends started in the range 290C° to 320C°. Above 420C° the major weight loss was completed. With the further increase in temperature there was a negligible weight loss which continued till 800C° in present case.
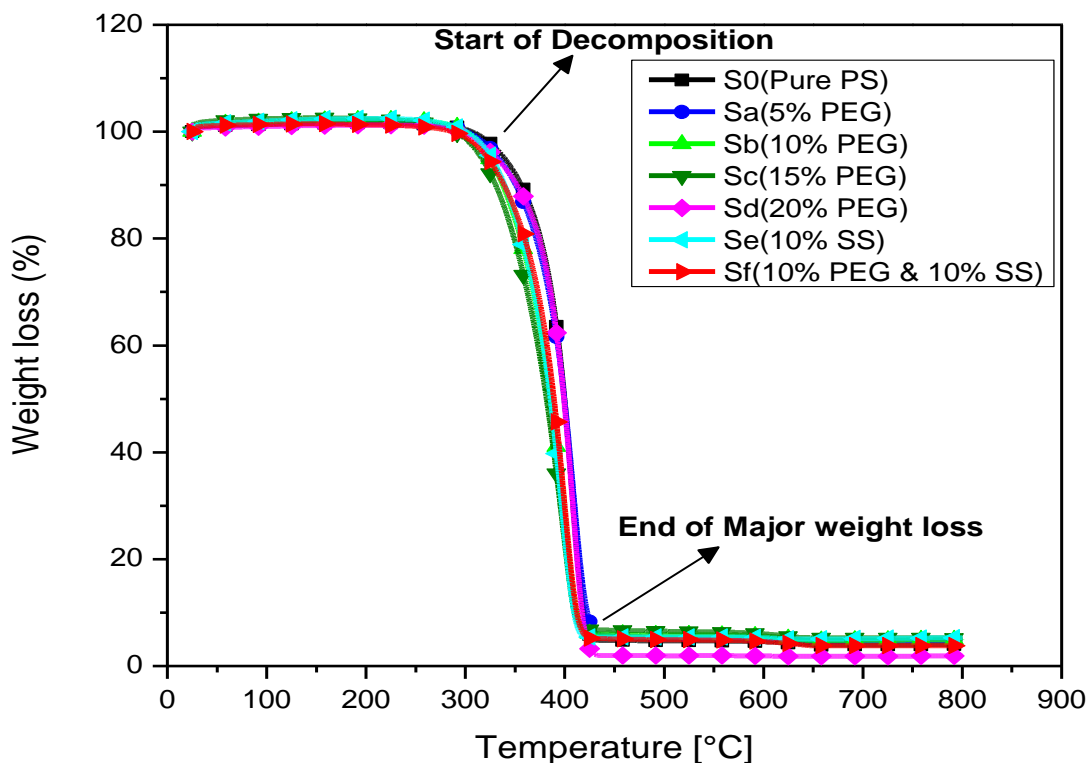

Fig 20: TGA Graphs of Various Polymer Blends

4.4.2 Initial Decomposition Temperature

The complete detail of weight loss and decomposition phenomena of various samples are given in table (12). The table shows that weight loss started at 305C° for pure Polystyrene. As PEG is added in Polystyrene blends the decomposition process started little earlier at 303C°, 299C°, 301C° and 297C° corresponding to 5% - 20% PEG blends respectively. It can also be observed that the difference is not too obvious. There is only a difference of

8C° within these five samples. So PEG managed to reduce the initial decomposition temperature of present blends by only few degrees. The decomposition was delayed in case of Se and Sf. The initial decomposition temperature of these two samples is even higher than Polystyrene, which means enhanced stability due to presence of Silver sulfadiazine.

4.4.3 Twenty Percent Weight loss

The similar results can be seen for 20 percent weight loss. Among Polystyrene and PEG based blends, the former proved to be more stable. More than 20% of weight loss occurred at 374C° for PS while the same amount was lost for Sd at 349C°. This shows that as the amount of PEG is increased in polymer blend, the decomposition temperature has decreased. There was a difference of 25C° for 20% weight loss between Polystyrene and 20% PEG based blend. The decomposition temperatures of Silver sulfadiazine based blends were comparable with Polystyrene.

4.4.4 Forty Percent Weight loss

Forty percent weight loss occurred at 393C° for Polystyrene. The same amount of decomposition occurred at 378 C°, 380 C°, 376 C° and 374 C° for Sa, Sb, Sc and Sd respectively. So PEG tend to reduce the thermal stability of various blends in comparison with Polystyrene by 19 C°. It can also be observed that the difference between these blends is very minimal i.e. 6 C°. The last two blends again proved to be most stable ones due to presence of Silver sulfadiazine.

4.4.5 Fifty Percent Weight loss

Half of the weight lost occurred at almost 399 C° for Polystyrene. The same weight loss was observed at 386C°, 387C°, 385C° and 382C° for 5-20% PEG blends respectively. These numbers prove that PEG when added tend to reduce the thermal stability of Polystyrene blends. So 20% PEG in Sd reduced the thermal stability of Polystyrene by

17C°. It can also be observed that the difference between PEG based blends is now reduced to 5C°. The last two blends showed maximum thermal stability due to presence of Silver sulfadiazine in them. The half of the material was lost at 400C° and 399C° for Se and Sf respectively.

4.4.6 Sixty Percent Weight loss

Table (12) shows that more than 60 percent of weight lost occurred at almost 404C° for Polystyrene. The same amount of decomposition was observed at 392C°, 393C°, 391C° and 389C° for Sa, Sb, Sc and Sd respectively, as the amount of PEG was increased from 5% to 20%. Silver sulfadiazine provided the maximum thermal stability to Polystyrene. The same amount of weight was observed at 404C° for both Se and Sf respectively.

4.4.7 Eighty Percent Weight loss

Table shows that 80 percent of Polystyrene decomposed at almost 404C°. The same amount of material was decomposed at 404C°, 405C°, 403C° and 401C° for Sa, Sb, Sc and Sd respectively, as the amount of PEG was increased from 5% to 20%. The table shows the difference of only 11C° between first five samples including Polystyrene. The difference between various PEG based blends is only 4C°. When Silver sulfadiazine was added with Polystyrene, the same amount of decomposition occurred at 413C° and 415C° for Se and Sf respectively. Silver sulfadiazine again proved to be more stable in comparison with PEG and Polystyrene.

Table 12: Decomposition Temperature Data of Various Polymer Blendsy

Sample Type	Temperature (C°) Corresponding To					
	Wt. loss Start	20%Wt loss	40%Wt loss	50%Wt loss	60%Wt loss	80%Wt loss
S0(Pure PS)	305	374	393	399	404	412
Sa (5% PEG)	303	357	378	386	392	404
Sb 10% PEG)	299	360	380	387	393	405
Sc (15% PEG)	301	356	376	385	391	403
Sd (20% PEG)	297	349	374	382	389	401
Se (10% SS)	317	376	394	400	404	413
Sf(10%PEG&10% SS)	312	371	393	399	404	415

4.4.8 General Conclusions

The initial Weight loss occurred at almost 297C° to 317C° range. The most of the material was decomposed between 401C° and 415C°.The major weight loss occurred between 350C° and 450C°.

The results show that PEG tends to decrease the thermal stability of Polystyrene blends. Addition of PEG decreased the thermal stability of Polystyrene blends by a few degrees which is acceptable. PEG is actually a soft low melting solid, so it reduced the thermal stability of blend due to synergetic effect of both ingredients. The results also show that different PEG based blends decomposed at different temperatures depending upon the amount of PEG. The minimum temperature at which decomposition started was 297C° for 20% PEG based blend. This temperature is still very high in comparison with room temperature at which most of the polymers are practically used. Polymer blends are not designed for high temperature applications just like metals. Any decomposition would be above 250C°, so PEG based blends are safe to use at room temperature. TGA results show that PEG does not affect the thermal stability of Polystyrene much.

On the other hand Silver sulfadiazine actually increased the thermal stability of Polystyrene blends by a few degrees. SS is itself thermally stable with high melting temperature so it brought the stability to its blends as well. The tri phase blends (PS/PEG/SS) tend to decompose by few degrees earlier when compared with PS/SS blends in most of the cases as shown by table. This is due to thermal instability of PEG which played its role. The difference of decomposition temperatures between Se and Sf is very minimal and ignorable. So in all of the samples discussed so far the thermal stability was not at stake in any case. All of the polymer blends are thermally stable till 280C° which is well above room temperature.

4.5 Differential Scanning Calorimetry (DSC)

DSC was done to check the thermal transitions of various polymer blends. DSC can tell us various transitions like glass transition, melting, crystallization, oxidation etc. but we are interested more in Tg. Most of the polymers are designed with respect to their glass transition as polymers properties start changing above glass transition temperature. Fig (21) shows the DSC thermographs of various polymer blends. The samples are scanned upto150C°.
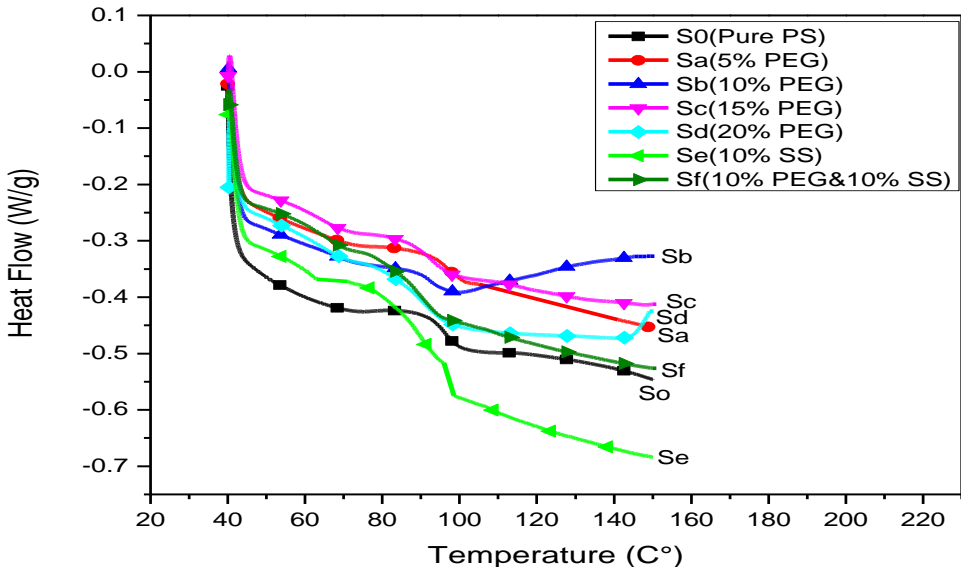
Fig21: DSC Thermographs of Various Samples

Table (13) shows Tg values of various samples calculated from DSC plots. The glass transition temperature of Polystyrene is around 90C°. As the amount of PEG is increased, Tg starts decreasing. The decreasing trend is 88 C°, 87 C°, 85 C° and 83 C° for polymer blends corresponding to 5%, 10%, 15% and 20% PEG respectively. This trend shows that as the amount of PEG is increased, glass transition temperature has decreased. This is due to the unstable nature of PEG whose transitions temperatures are quite low in comparison with Polystyrene. Synergetic effect of thermally unstable Poly (ethylene glycol) managed to decrease the Tg by only a few degrees. The decrease in transition temperature is not so prominent due to maximum amount of Polystyrene present in the blends.

Silver sulfadiazine has almost no effect on glass transition temperature with the Tg value of 89 C° corresponding to Se with 10% SS. The reason is that SS is thermally stable just like PS. The neutral role of SS is also due to its non-polymeric nature and lesser amount present in the blend. All of the blends discussed so far can be used for room temperature applications because their glass transition temperatures are well above room temperature. Blending did not affect much the thermal transition of Polystyrene.

**Table 13:** Glass Transition Temperature (Tg) of Various Samples

Sample Name	Glass Transition Temperature Tg (C°)
S0(PS)	90
Sa(5% PEG)	88
Sb(10%PEG)	87
Sc(15%PEG)	85
Sd(20%PEG)	83
Se(10%SS)	89
Sf(10%PEG&10%SS)	87

4.6 UV Visible Spectroscopy

Fig (22) shows the UV-Visible spectrographs of various samples. The samples are scanned between Ultra violet and visible portions of electromagnetic spectrum. The sample most disturbed by anti-algal blend would have clear zone of inhibition which would allow maximum amount of light to pass through, reducing the absorbance to minimum. The undisturbed sample would have maximum amount of algal growth, so it would not allow light to pass through easily. The maximum amount of light would be absorbed in that case.

The anti-algal behavior of first three samples (S0, Sa & Sb) was tested against Dictyosphaerium sp. strain HM1 (DHM1), the other two (Sb & Sc) against Dictyosphaerium sp. strain HM2 (DHM2) and the remaining two (Se & Sf) against Pectinodesmus sp. strain HM3 (PHM3). The samples created their respective inhibition zones depending upon their strength and composition. Then all of the samples were undergone through UV-Visible Spectroscopy.

Fig (22) indicates that pure Polystyrene sample absorbed maximum amount of spectrum. The absorbance in this case is maximum. The curve corresponding to Sa shows less absorption which continues the decreasing trend in Sb, Sc, Sd, Se and Sf. The curve corresponding to Sb is below Sa which comparatively shows less absorption. The spectrographs of Sc and Sd are further below which allow more amount of light to pass through in comparison with Sb. The Se based curve shows the second minimum absorption of UV- visible spectrum. The curve corresponding to Sf is at the lowest location in the plot with minimum absorbance.
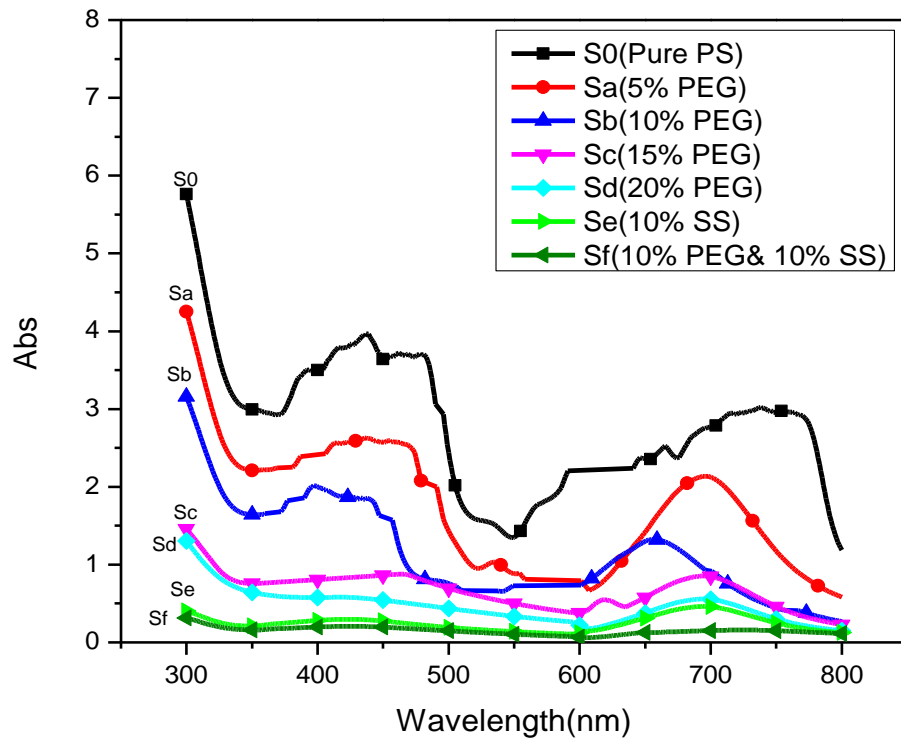
**Fig 22:**UV-Visible Spectrographs of various Polymer blends

The graph clearly shows the anti-algal effect of various blends. As the anti-algal agent is added in the blend, the absorption decreases which indirectly tells us about the anti-algal effect of respective samples. The absorption is low when algal growth is inhibited and there is clear area for light to pass through. The plots indicate that as the amount of PEG is increased, the absorbance is decreased which means anti-fouling effect becomes more pronounced. So the curves indicate the indirect relationship between amount of PEG and its absorbance which decreases with the increase in amount of PEG.

The graph also shows the anti-algal effect of Silver sulfadiazine. The curves corresponding to SS shows minimum absorption. These results show that SS is a better anti-algal agent than PEG. The curves also support the disk diffusion results which can indirectly tell us about the width of inhibition zone and effectiveness of anti-fouling blend. They also show the effectiveness of PEG against SS. The overall pattern of graphs also remained same.

4.7 Zeta Potential Measurements

Zeta potential is calculated using a combination of measurement techniques like Electrophoresis and Laser Doppler Electrophoresis. This method measures the velocity of certain particle in a respective suspension against applied electrical field. Sample viscosity is known in advance or it can be calculated. The dielectric constant can also be found from literature. Using all the above mentioned parameters, Zeta potential can be calculated. But the current equipment can itself calculate zeta potential factor using the automated software following same procedure. It can also calculate a number of other parameters like particle size, poly dispersity index etc.

Fig (22) shows the zeta potential graphs of three different types of algal strains namely Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3) against total number of counts. The overall pattern of all the plots is same. Figure shows that there is not much difference between zeta potential of different algal strains, though there is difference in number of counts.
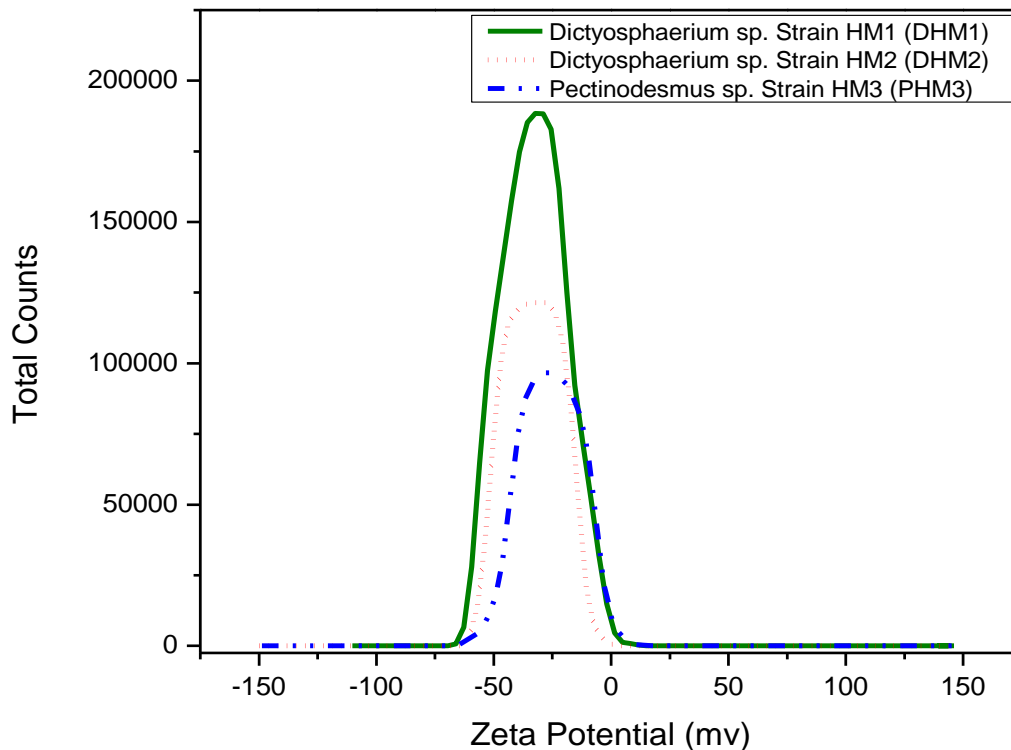
Fig 22: Zeta Potential of Different Algal Strains

Table (14) lists different parameters calculated by the equipment like:

4.7.1 Zeta Potential (mV)

Zeta potential of particles was measured in milli volts. Zeta potential values are negative for all algal strains which show that their particles are negatively charged. There is not much difference between zeta potential values for various Strains. Calculation of this charge is important because charge effects various parameters like surface properties, hydrophilicity or hydrophobicity, contact angle, adhesion etc. All of these factors are important in defining anti-fouling properties and thus devising certain anti-fouling system.

4.7.2 Particle Size (d.nm)

Z.Ave is actually the average diameter of respective algal strains in nano meters. Table shows that there is difference in particle size of various algal strains. This particle size can actually effect various parameters calculated by Zeta sizer i.e. mobility, Poly dispersity Index etc.

4.7.3 Poly Dispersity Index

Poly Dispersity index explains homogeneity and heterogeneity of dispersion. PDI values of current experiment show that Pectinodesmus sp. strain HM3 (PHM3) suspension was more homogenous than Dictyosphaerium sp. strain HM2 (DHM2) and Dictyosphaerium sp. strain HM1 (DHM1) which was most heterogeneous among the three. The homogeneity of Dictyosphaerium sp. strain HM2 (DHM2) lies in between the other two strains.

4.7.4 Mobility (μmcm/Vs)

Particles move towards the electrode of opposite charge, their velocity is measured and expressed in unit field strength as their mobility. The mobility of that Pectinodesmus sp. strain HM3 (PHM3) particles was maximum followed by Dictyosphaerium sp. strain HM1 (DHM1) and Dictyosphaerium sp. strain HM2 (DHM2).

4.7.5 Conductivity (mS/cm)

Cond stands for conductivity of colloidal dispersion. Table shows that, although there is not much difference in conductivity, Pectinodesmus sp. strain HM3 (PHM3) is most conductive in comparison with Dictyosphaerium sp. strain HM1 (DHM1) and Dictyosphaerium sp. strain HM2 (DHM2). This conductivity is linked with surface properties most importantly surface charge, which are directly associated with Bio-fouling process. Conductivity values of different strains can thus help in synthesizing certain anti-fouling system.

**Table 14:** Different Factors Calculated by Zeta-Sizer for various Algal strains

Sample Name	ZP(mV)	Z.Ave (nm)	PDI	Mob (µmcm/ Vs	Cond (mS/cm)
Dictyosphaerium sp. strain HM1 (DHM1)	-32.7	3.29E+04	0.286	-2.562	0.335
Dictyosphaerium sp. strain HM2 (DHM2)	-33	1.88E+04	0.437	-2.59	0.278
Pectinodesmus sp. strain HM3 (PHM3)	-25.7	1.29E+04	1	-2.014	0.426

4.8 Dilatometry

Fig (23) shows the dilatometry plots of various samples. Percentage linear change is measured against temperature. It can clearly be seen that no linear change occurred for any sample below 90C° and polymers are practically used at room temperature. As far as application of polymers blends is concerned, there are no problems of thermal expansion at room temperature. The main thermal transitions occur above 90C° for all of the blends which means thermal stability at room temperature. The glass transition temperature obtained from DSC is also around 90C°. Se with 10% SS is the most thermally stable sample with minimum thermal expansion due to presence of Silver sulfadiazine and Polystyrene which are both thermally stable with high melting points, so the resultant blend should be stable. S0 is the next sample in the list followed by Sf which show some

percentage linear change. In the next set of samples PLC either increases or decreases with temperature showing some thermal expansion. This is due to the presence of PEG-1000 an unstable polymers, which somehow effects thermal stability of blends.

It is important to mention that all of the samples show negative linear change at high temperatures which means shrinkage or contraction of materials. At high temperatures polymer chains may intermingle with each other, the chains don't find enough space to move away from each other, so dimensions of the samples decrease as a result. Sc and Sd show negative amounts of linear changes significantly in the beginning due to presence of more amount of unstable PEG comparatively which also causes more chains entanglement. It can also be proved from the plots that as the amount of PEG increases, PLC also increases because the amount of nonstable component actually increased.
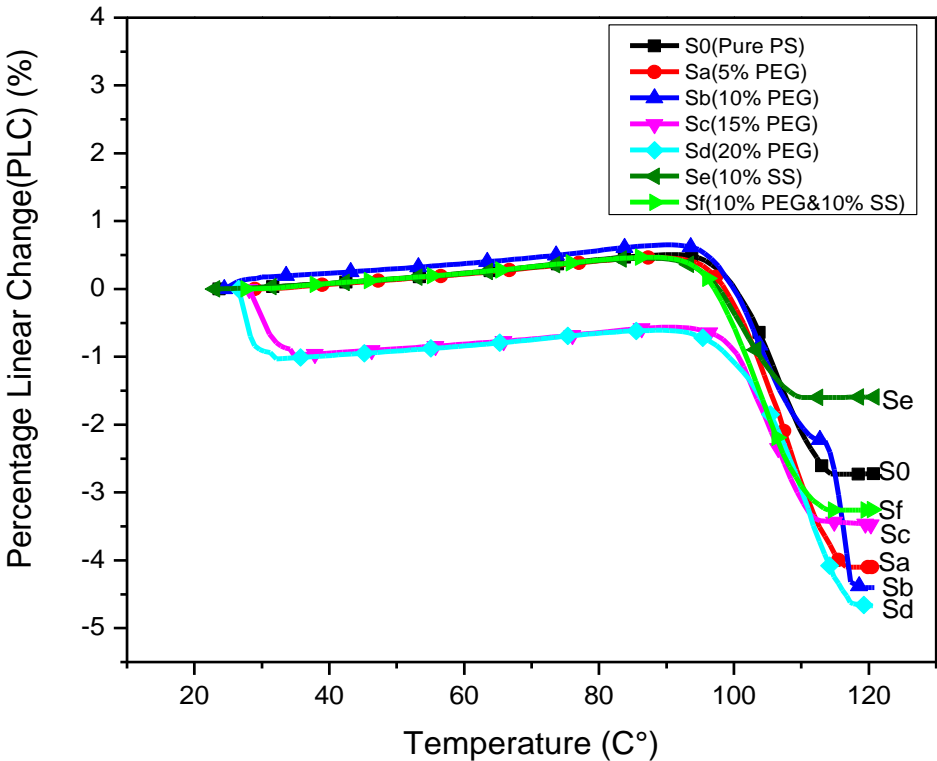
Fig 23: Dilatometry Plots of various Polymer blends

Chapter 5

CONCLUSIONS & RECMMENDATIONS

5.1 Conclusions

1. Poly (ethylene glycol) (PEG-1000) and Silver sulfadiazine (SS) are excellent anti-fouling agents as far as their susceptibility to inhibit microalgae is concerned.

2. The agar disk diffusion method was employed to check the inhibition of algal growth against Dictyosphaerium sp. strain HM1 (DHM1), Dictyosphaerium sp. strain HM2 (DHM2) and Pectinodesmus sp. strain HM3 (PHM3) which proved that SS is a better anti-fouling agent in comparison with PEG-1000. Ternary blend of PEG, SS and PS was the most effective one due to presence of both strong anti-algal agents, followed by PS/SS one. PEG/PS based polymer systems were also quite effective.

3. The anti-algal studies indicate the direct relationship between amount of anti-algal agent and its inhibition to microbial growth. The results of agar disk diffusion were further authenticated through optical microscopy and UV-visible Spectroscopy which proved the presence of inhibition zone in all of the blends.

4. Mechanical properties tested through UTM showed reasonable tensile profile of blends to be used in certain application. The thermal properties were studied using DSC, TGA and Dilatometry. The thermographs showed that blends are thermally stable enough with appropriate thermal properties.

5. The charge on various algal strains was calculated using zeta potential measurements which also gave particle size and other parameters of algal suspensions depending upon their composition. The resultant anti-fouling blends can be used for a number of

applications like food processing, water purification, bio corrosion, photo-bioreactors etc.

5.2 Future Prospects & Recommendations

1. PS/PEG/SS blends can be tested for their Anti-bacterial and Anti-fungal properties and a comparison can be generated with respective anti-algal behavior of same blends.

2. Application of Polymer blends in certain Practical field.

3. High Molecular Weight grades of PEG can be tested to study the relationship between molecular weight and Anti-Fouling properties in this particular case.

4. Increasing the concentration of Anti-algal agents & observing their new anti-microbial effect.

5. Using some other Matric like PVC with PEG-1000 & SS and studying its Anti-algal effect

6. Using of some other Polymer system instead of PEG-1000 and comparing its anti-microbial properties with it.

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