A comparative study on the bioplastic production from *Spirulina platensis* Gomont, *Manihot esculenta* Crantz and *Manihot esculenta* Crantz with addition of *Spirulina platensis* Gomont.

A dissertation Submitted to
MAHATMA GANDHI UNIVERSITY
KOTTAYAM – 682562

For the award of the degree of
MASTER OF PHILOSOPHY
IN
SCIENCE (BOTANY)

By
GANGA JOHNY
(Reg. No. **SMP20BOT005**)

Department of Botany and Centre for Research
St. Teresa's College (Autonomous)
Ernakulam
Kochi – 682011
2021-2022

MAHATMA GANDHI UNIVERSITY

CERTIFICATE ON PLAGIARISM CHECK

1.	Name of the Research Scholar	**GANGA JOHNY**
2.	Title of the Thesis/Dissertation	A Comparative Study on the Bioplastic Production from Spirulina Platensis Gomont, Manihot Esculenta Crantz and Manihot Esculenta Crantz with Addition of Spirulina Platensis Gomont.
3.	Name of the Supervisor(s)	Dr. Elsam Joseph
4.	Department/Institution/ Research Centre	Department of Botany St. Teresa's College (Autonomous) Ernakulam
5.	Similar Content (%) identified	4% **(Four)**
6.	Acceptable Maximum Limit	25%
7.	Software Used	Ouriginal
8.	Date of Verification	05-07-2022

**Report on plagiarism check, items with % of similarity is attached*

Checked by (with Name, Designation & Signature) :

DL
05/07/22

LETHA ARAVIND
Assistant Librarian Gr.- I in-charge
M.G. University Library
P.D. Hills P.O., Kottayam - 686 560

Name & Signature of the Researcher* : Ganga Johny

Ganga
05/07/22

Name & Signature of the Supervisor(s) : Dr. Elsam Joseph

Dr. ELSAM JOSEPH
Associate Professor
Research Guide
Department of Botany
St.Teresa's College (Autonomous)
Ernakulam - 682 035

Name & Signature of the HoD/ HoI (Chairperson of the Doctoral Committee) :

Dr. Stephen Sequeira M.Sc.,Ph.D
Assistant Professor
Post Gradu [illegible]
Maharaja's College
Ernakulam - 682011

Curiginal

Document Information

Analyzed document	GANGA JOHNY-A comparative study on the bioplastic production from Spirulina platensis Gomont Manihot esculenta Crantz and Manihot esculenta Crantz with addition of Spirulina platensis Gomont.pdf (D141736207)
Submitted	2022-07-05 08:47:00
Submitted by	Smt. Letha Aravind
Submitter email	library@mgu.ac.in
Similarity	4%
Analysis address	library.mgu@analysis.urkund.com

Sources included in the report

W

URL: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1073.9153&rep=rep1&type=pdf
Fetched: 2022-06-03 14:40:58

DR
05/07/22

LETHA ARAVIND
Assistant Librarian Gr.-I in-charge
M.G. University Library
P.D. Hills P.O., Kottayam - 686 560

DECLARATION

I hereby declare that the dissertation entitled **"A comparative study on the bioplastic production from *Spirulina platensis* Gomont, *Manihot esculenta* Crantz and *Manihot esculenta* Crantz with addition of *Spirulina platensis* Gomont''** submitted is an authentic record of the research carried out by me, under the supervision and guidance of Dr. Elsam Joseph, Associate professor, Department of Botany, St. Teresa's College (Autonomous) Ernakulam, in partial fulfilment of the **M. Phil** degree of Mahatma Gandhi University, Kottayam, and that no part of this thesis has been presented before for any degree, Diploma, associateship or other recognition from any university.

Ernakulam
July 2022

GANGA JOHNY
Department of Botany
St.Teresa'sCollege(Autonomous)
Ernakulam, Kochi-682035
(Reg. No. SMP20BOT005)

CERTIFICATE

This is to certify that the dissertation entitled "**A comparative study on the bioplastic production from *Spirulina platensis* Gomont, *Manihot esculenta* Crantz and *Manihot esculenta* Crantz with addition of *Spirulina platensis* Gomont.**" Submitted by **Ganga Johny** (Reg.No. SMP20BOT005) for the degree of **Master of Philosophy** to **Mahatma Gandhi University, Kottayam** is a record of bonafide research work carried out by her under my direct guidance and supervision.

I considered that the work has reached the standards and fulfilling the requirements of the rules and regulations relating to the nature of the degree. The contents embodied in the thesis have not been submitted for the award of any other degree or diploma in this or any other university.

Ernakulam
July 2022

Dr. ELSAM JOSEPH
Associate professor
Department of Botany
St. Teresa's College
(Autonomous)
Ernakulam, Kochi - 682035

CERTIFICATE

This is to certify that the dissertation entitled "**A comparative study on the bioplastic production from *Spirulina platensis* Gomont, *Manihot esculenta* Crantz and *Manihot esculenta* Crantz with addition of *Spirulina platensis* Gomont.**" Submitted by **Ganga Johny** (Reg. No. SMP20BOT005) for the degree of **Master of Philosophy** to **Mahatma Gandhi University, Kottayam** is a record of bonafide research work carried out by her under guidance and supervision of Dr. Elsam Joseph, Associate Professor, Department of Botany.

I considered that the work has reached the standards and fulfilling the requirements of the rules and regulations relating to the nature of the degree. The contents embodied in the thesis have not been submitted for the award of any other degree or diploma in this or any other university.

Ernakulam
July 2022

Liza

Dr. Liza Jacob
Assistant professor
Head of the Department
Department of Botany
St. Teresa's College
(Autonomous)
Ernakulam, Kochi - 682035

Valid
13/7/2022.
Dr. Stephen Sequeira.

ACKNOWLEDGEMENT

It gives me immense pleasure to present the completion of this dissertation. This would not have become a reality without the guidance and support of many people.

First of all, I am greatly indebted to my guide Dr. Elsam Joseph, Associate Professor Department of Botany, St.Teresa's College (Autonomous) Ernakulam, for her love, support, constant motivation, immense knowledge and patience which have been instrumental in helping me at every step throughout my work.

I owe my heartfelt gratitude to the Principal of the College Dr. Lizzy Mathew for granting me the golden opportunity to pursue my studies and permitting me to make use of the resources of the College.

My sincere thanks go to the Head of the Department of Botany, Dr. Liza Jacob for her timely guidance and help during the course of my project. I owe special thanks to Dr. Arya P Mohan for the support and inspiration during the course of the study.

A word of thanks goes to Dr. Sunil N. Kutty, Head of the Department of Polymer Science and Rubber Technology, Cochin University of Science and Technology (CUSAT) for the guidance and assistance in completing this work on time and efficiently. I wish to express my gratitude to Sophisticated Test and Instrumentation Centre (STIC) CUSAT, Kalamassery for helping me to complete my work on time. I also wish to express my gratitude to Central Marine Fisheries Research Institute (CMFRI) for the guidance in completing this work efficiently.

My friends and other research scholars also deserve a word of appreciation for their great help and support in my journey. And very warm appreciation to the non-teaching staff of the Department who have rendered much help and support during the course of my work.

Very special gratitude from the bottom of my heart goes to my Father Johny V.V, my mother Saritha Johny and my sister Dhanya Johny for their moral support. Above all, I thank God almighty for the showers of blessings poured down on me in successfully completing my task and help to set another milestone in my life.

Ganga Johny

ABSTRACT

Bioplastic is one among the alternative to reduce environmental problems caused by petroleum-based plastics. The bioplastics are produced from renewable resources like Corn starch, Cassava starch, or microorganisms under controlled conditions. This work aims at the development of the bioplastic sheets from *Spirulina platensis* (*S. platensis*), *Manihot esculenta* (*M. esculenta*) and *M. esculenta* with addition of *S. platensis*. *S. platensis* is cultured in the Zarrouk medium, collected and stored after washing and drying. The effect of pH and modified Zarrouk`s medium on the growth rate of *S. platensis* strain was evaluated. It was noted that growth rate of *S. platensis* increases with increase in pH from 8 to 9.5.The bioplastic sheet was made from *S. platensis*, *M. esculenta* and *M. esculenta* with addition of *S. platensis* using glycerol as plasticizer. The major aspects of the comparative study of bioplastics include the biodegradability, moisture absorption, Tensile strength and SEM analysis. The result shows that the moisture absorption and biodegradability of bioplastic was high in bioplastic prepared using *M. esculenta* with acetic acid. The presence of hydroxyl (OH), carbonyl (CO) and ester (COOH) groups in Cassava starch indicates that concentration of hydrophilic properties in the Cassava starch based bioplastic is high. SEM images show that bioplastic prepared using Cassava with acetic acid has a better consistent surface in compared to bioplastic using *Spirulina* and Cassava with calcium carbonate. The tensile strength of bioplastic was high in bioplastic prepared using *M. esculenta* with calcium carbonate as a filler.

Key words: Bioplastic, *Spirulina platensis*, Zarrouk medium, *Manihot esculenta*, Biodegradation, Moisture absorption, Tensile strength, SEM analysis.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

A	Alpha
ASTM	America Society for Testing and Material
$CaCl_2.2H_2O$	Calcium chloride dihydrate
CMFRI	Central Marine Fisheries Research Institute
CMT	Chip Multi Threading
Co2	Carbon dioxide
CUSAT	Cochin University of Science and Technology
$CuSO_4.5H_2O$	Copper sulphate pentahydrate
°C	Degree Celsious
DSC	Differential Scanning Calorimerty
K2HPO4	Dipotassium phosphate
EDTA	Ethylene Diamine Tetra Acetic acid
Eg	Exempli gratia
FeSO4.7H2O	Ferrous sulphate heptahydrate
Gm	Gram
$C_3H_8O_3$	Glycerol
DNA	Deoxyribo Nucleic acid
HCMV	Human Cyto Megato Virus
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
Lux	Luminous flux
$MnCl_2.4H_2O$	Manganese dichloride tetrahydrate
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
Mg	Milligram
Ml	Millilitre
MPa	Mega Pascal
MCC	Micro Crystalline Cellulose
mm	Millimetre
nm	Nanometre

H_3BO_3	Orthoboric acid
%	Percentage
PE	Poly Ethylene
PEG	Poly Ethylene Glycol
PHAs	Poly Hydroxy Alkanoates
PHB	Poly Hydroxy Butyrate
PLA	Poly Lactic Acid
pH	Potential of Hydrogen
K_2SO_4	Potassium sulphate
PP	Poly Propylene
PS	Poly Styrene
$NaHCO_3$	Sodium bicarbonate
NaCl	Sodium chloride
Na_2MoO_4	Sodium molybdate
NaNO3	Sodium nitrate
SEM	Scanning Electron Microscopy
STIC	Sophisticated Test and Instrumentation Centre
VOCs	Volatile Organic Compounds
$ZnSO_4.4H_2O$	Zinc sulphate tetrahydrate

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

INTRODUCTION

Today, we can’t imagine a world without plastics. Plastic has a significant role in day to day life including the biomedical applications. It is commonly used as covers, pipe, bottles, cookware etc. The polymer such as Polyhydroxyalkanoates (PHAs) has wide range of applications in medical field. It has the potential to generate medical devices such as rivets, tacks, stents, staples etc. The Bakelite was the first synthetic plastic which appeared in the 20th century. It was invented by Leo Baekeland in New York in 1907. After that, second synthetic plastic was developed in 1911 by the development of ‘rayon’. The wide spread use of plastic was occurred after Second World War (Geyer *et al.*, 2017).

The plastics are mainly made from non-renewable polymers like Poly Styrene, Poly Propylene and Poly Ethylene. The polypropylene is the most commonly used source due to its chemical stability and non polar nature. The hydrocarbons as monomers linked together to form long chain Polymers. The process of forming polymers from single monomer unit called polymerization. There are mainly two types of polymerization reaction. They are condensation polymerization and addition polymerization. The addition polymerization is the joining of two identical monomers. Polyvinyl chloride, polystyrene and polypropylene are examples of polymers formed by addition polymerization reaction. The joining of two or different monomers having different length is called as condensation polymerization. The examples for polymers formed by condensation polymerization are nylon, polyester and polyurethane. There are some additives (chemicals used to increase the performance) used in preparation of plastics. They are plasticizer, colorants, heat stabilizers, antioxidants, fillers, compatibilizer and blowing agents. Additives procure the properties of plastics such as flexibility, colour and desired scheme (Zhong *et al.*, 2018).

The word plastic comes from two words ‘plasticus’ (latin) and ‘plastikos’ (greek) which means ‘molded. Plastic production has been increasing at enormous rates. The production rate has increased substantially over the last 80 years from around 0·5 million tonnes to 280 million tonnes at present. Plastic has many useful properties. Plastic do not rust like metals and rot like wood or paper. They can be easily produced in any colour by using additives. The density of plastic is lower than metal. Plastics can be easily molded and shaped according to our need (Kumar *et al.*, 2020).

The natural degradation of plastic is a slow process due its chemical configuration (Ganguly, 2018). The pyrolysis is the one and only thermal treatment to destruct plastic material at an elevated temperature (Geyer *et al*., 2017). They produce poisonous fumes during burning time. The now a days the quantity of abandoned plastic waste is increasing hence the environmental impact is increasing (Duranay *et al*., 2019). It is impossible to recover all discarded plastic materials by recycling (Amaf, 2011). The Volatile Organic Compounds (VOCs) are released during recycling process. Plastic recycling process also generate carbon molecule which imparts more environmental problems like Global warming. So, the recycling of plastic products is not a solution for the plastic pollution. One of the alternatives to reduce the plastic waste is to replace the production with bioplastic (Zeller *et al*., 2013).

1.1 Importance of Bioplastic

The bioplastics are degradable by microorganisms, derived from renewable biomass. The main advantage of bioplastic production is their contribution to reduce greenhouse gas emission as it is biodegradable and environment friendly organic product (Demirbas, 2007). The microorganisms after the decomposition of bioplastics can produce water, carbon dioxide and methane (Wahyuningtyas and Suryanto, 2017). The bioplastic production consumes 65% less energy compared to the petrochemical plastics (Bezirhan and Ozsoy, 2014). The soil microorganisms decompose bioplastics without generating harmful by products (Sreenikethanam and Bajhaiya, 2021). The biodegradable plastics can degrade 10 to 20 times faster than synthetic polymers. Plastic based on starch is safe for the environment (Sofiah *et al*., 2019). Photodegradable plastics are sensitive to light. The extensive ultra violet can disintegrate the polymeric structure of bioplastic (Bezirhan and Bilgen, 2015).

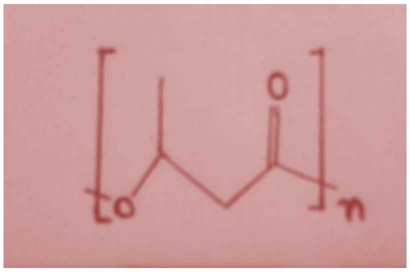
Apart from the numerous advantages of bioplastic, they do have some drawbacks as well. The making process of bioplastic is expensive compared to petroleum based plastic production. The bioplastic have poor mechanical properties and high level of moisture content (Maulida *et al*., 2016). Some bioplastic have very shorter life span compared to fossil fuel based plastics due to weaker mechanical properties. Soil and water pollution may result due to the improper and uncontrolled disposal of the bioplastic wastes. Unusable products may generated by the mixing bioplastics into other petroleum based plastics while recycling (Bezirhan and Bilgen, 2015).

1.2 Sources for bioplastic production

The raw materials for bioplastics originate from natural components such as polysaccharides (example: starch, cellulose, chitin and lignin), proteins (example:gelatine, casein and wheat gluten) and lipids (example: plant oils and animals fats) (Maulida *et al*., 2016) .The Microalgal biomass is one among the raw material for the production of bioplastics. Algae are photosynthetic eukaryotic organisms. They are photosynthetic with fast growing ability (Jegan *et al*., 2014). They are rich in aminoacids, proteins and lipids (Wells *et al.,* 2017). According to Rahman and Miller (2017), they can be use directly or indirectly (secondary metabolites). The various bioplastics that can be made from algal feedstock include Hybrid plastics, Cellulose-based plastics, Poly-Lactic Acid (PLA) and Bio-Poly Ethylene (PE) based plastics (Amaf, 2011). The protein present in the algae is the most important factor that contributes the formation of polymers (Dianusranti *et al*., 2018). The proteins are formed by the condensation reaction of aminoacid molecule. The main advantage of using microalgae for bioplastic production is its ease of cultivation. It does not take a long time and large area for cultivation. The microalgae also have the potential to reduce high amount of CO2 in the atmosphere through oxygenic photosynthesis (Dianursanti *et al*., 2018).

The PolyHydroxy Butyrate (PHB) content of algal biomass enhances the recyclable property of plastic. PHB belongs to a family of polyesters called PolyHydroxy Alkanoates (PHAs). PHAs are produced in microorganisms as a source of energy (Abdo and Ali, 2019). The mechanical properties of PHB resemble those of commodity plastics such as Poly Ethylene (PE), Poly Propylene (PP) and Poly Styrene (PS) (Jangra *et al*., 2016). Algae produce PHB as an intracellular energy and carbon storage molecule. The PHB production is more triggered in nutrient starved condition (McAdam *et al*., 2020). The bacteria also produce PHB as its food storage material. The PHB production of an organism is induced by α- ketothiolase enzyme (Amaf *et al*., 2011). Sayeda *et al*., (2019) reported that addition of sodium acetate to the cultivation media of the selected algal strains affected the production of PHB. According to Monshupanee *et al*., (2016) intracellular PHB production and accumulation using microalgae can be optimized using the amount of acetate supply, varying light and nutrient conditions.

Figure 1: Structure of PHB



According to Önen *et al.*, (2020) many different microalgae have the potential to produce bioplastic. *Spirulina* is one among the algae that having the intracellular PHB production ability that can be utilized to produce bioplastic. *Spirulina* is a filamentous, photosynthetic, edible Cyanobacterium. It grows vigorously in strong sunshine under high temperatures and high alkaline conditions. It has very high macro and micronutrient contents. The major nutrients include calcium, niacin, iron, vitamins, magnesium and potassium (Costa *et al.*, 2019). It contain approximately 65% of protein and all essential aminoacids. The non essential amino acids like alanine, aspartic acid and cysteine are also present. *Spirulina* possess a wide range of coloured compounds such as carotenoids, chlorophyll and phycobiliproteins. phycocyanin, allo-phycocyanin and phycoerythrin are the principal phycobiliproteins (Dineshkumar *et al.*, 2015).

Spirulina requires high amount of nutrients and salt concentration for their effective growth (Soni et al., 2019). Different media have been used for culturing *Spirulina* such as Zarrouk's media, Rao's media and Revised media (Dineshkumar *et al.*, 2015). The successful and standard medium used for culturing *S. platensis* is Zarrouk's medium. *Spirulina* forms massive populations in high alkaline conditions, pH up to 11. The salinity of the medium has the major role in the dominance of algae than alkalinity (Amara and Steinbüchel, 2013). The optimum temperature requires is 30-35ºC (Chandrasekaran *et al.*, 2015). The light intensities are required during the growth phase of *Spirulina*. The water quality also plays an important role in mass production of *S. platensis*. It will affect the solubility of nutrients in the medium and by the accumulation of heavy metals by algae during growth phase. The proper agitation is necessary for the better yield of *S. platensis*.

The advantage of agitation is the uniform distribution of carbon dioxide and thermal stratification (Soni *et al*., 2019).

The intracellular PHB accumulating property of *Spirulina platensis* (*S. platensis*) is high compared to the other *Spirulina* species such as *S. maxima* and *S. fusiformis* (Amaf, 2011). *Spirulina platensis* is a spiral Cyanobacteria that contain 50-70% protein content (Afriani *et al*., 2018; Christwardana *et al*., 2011). Its protein contain all essential amino acids. They occur naturally in tropical and subtropical lakes and has fast growing property (Amaf *et al*., 2011). *S. platensis* has high potential to act as filler in bioplastic composite production because of its high protein content (Zeller *et al*., 2013). Due to the small size of *S. platensis*, protein present in it is needed not to be extracted (Dianursanti *et al*., 2018).

Amaf *et al*., (2011) reported that biodegradable property of bioplastic using *Spirulina* biomass was high compared to commercial plastics. Dianursanti *et al*., (2018) reported that addition of glycerol as plasticizer in *S. platensis* for the bioplastic production resulted in an improvement in elongation properties of bioplastic. Plasticizer is a low molecular weight, non-volatile substance used as an additive to improve flexibility (Vieira *et al*., 2011). In the research by Kok Sheng *et al*., 2009 showed that, tensile strength of commercial plastic is similar to the bioplastic prepared with a compatitibilizer. Based on the research by Dianursanti *et al*., (2018) use of compatibilizer and plasticizer can improve the mechanical properties of bioplastic.

The bioplastic can be also made from vegetable oil, Jack fruit seed starch, Corn flour and Cassava starch (Krishnamurthy and Amritkumar, 2019). Cassava has greater contribution for the bioplastic production and has high amount of protein that enhances the binding property of bioplastics. Cassava (*Manihot esculenta*), belongs to Euphorbiaceae family. Cassava is extensively cultivated in tropical and subtropical regions as an annual crop for its edible starchy tuberous root. It contains essential nutrients such as carbohydrates, vitamin A, vitamin C, iron, calcium, phosphorous and phenolic compounds (Fathima *et al*., 2022). Cassava starch contain high amount of amylopectin (Amaraweera *et al*., 2021). Starch- based biodegradable plastics are sensitive to water and have high water vapor permeability (Santana *et al*., 2018). In order to overcome this, starch is further processed using plasticizers (Narancic *et al*., 2020).

The plasticizers are added to the bioplastic production to increase the flexibility and elasticity of bioplastic. Glycerol and Sorbitol are commonly used plasticizer for bioplastic production (Sofiah *et al.*, 2019). They give flexibility in the polymer structure by decreasing the intermolecular forces and glass transition of material. The addition of calcium carbonate as reinforcing filler will enhances the properties of bioplastic. Calcium carbonate has a great contribution to enhance the strength, durability and working properties of bioplastic (Abidin *et al.*, 2021).

Scanning Electron Microscopy (SEM) is a technique which is useful to observe the morphology of the bioplastic film in order to examine the interactions between different components in the composite (Önen *et al.*, 2020). SEM analysis gives the information about non gelatinized granules of starch. Biodegradable behavior of bioplastics can be determined using soil burial degradation test. The extent of damage of bioplastics can be analysed using degradation percentage. The several bacteria found in the soil such as *Pseudomonas* species, *Streptococcus* species, *Staphylococcus* species, *Bacillus* species, and *Moraxella* species has higher impact on the degradation of bioplastics (Wahyuningtiyas and Suryanto, 2017). The Meta bisuphite is useful for preventing the growth of Fungi and Bacteria on films (Chandarana and Chandra, 2021). The presence of glycerin in bioplastic film also influences the rate of degradation (Vinodh, 2021). The moisture absorption ability of bioplastic film is influenced by starch and glycerol concentration (Santana *et al.*, 2018). Tensile strength of bioplastic is its ability to withstand under higher pressure without breaking (Vinodh, 2021). The highest tensile strength to bioplastic film was observed in films which have lowest glycerol concentration (Santana *et al.*, 2018). The demand for biodegradable bioplastic is increasing due to the increasing difficulty of managing fossil fuel based plastics. Therefore several new researches are initiated in order to promote eco-friendly, biodegradable plastics. Further research is necessary to optimize the process for industrial applications.

CHAPTER 2

REVIEW OF LITERARTURE

REVIEW OF LITERATURE

Plastics are large family of polymers, generally made from fossil feedstocks. The global plastic production has been increasing as a result of global economic growth and improvement of living standards. The plastic is used as a packaging material for various purposes. They have lot of industrial applications too (Ismail *et al*., 2019). All plastic ever produced has not been recycled, hence it generating large volume of plastic waste. The accumulation of plastic wastes in soil would affect the penetration and dissipation of water and air into the earth (Selvamurugan and Pramasivam, 2019). The fossil fuel based plastic pose serious problem to aquatic and terrestrial ecosystem. They also affect the economic growth and environmental stability (Zeller *et al*., 2013). The conventional plastics made from crude oil are a diminishing natural source (Abdo and Ali, 2019). They preserve in soil for centuries due to the lack of microbial degradation. It is impossible to recycle all the discarded plastics in an effective way (Amaf, 2011). The demand for the polymer is increasing due to development in all aspects of life (Chan *et al*., 2020). The bioplastics can replace the fossil fuel based plastics in order to reduce environmental problems (Narancic *et al*., 2020).

The bioplastics are biodegradable plastics, which are derived from natural sources such as plant or microorganisms (Selvamurugan and Pramasivam, 2019). The soil microorganisms can degrade the bioplastics into carbon dioxide, methane, water, inorganic compounds, or biomass without generating any harmful substance to the environment. They lead to sustainable and circular economy (Sreenikethanam and Bajhaiya, 2021). The Biodegradable form of plastic was first characterized by French researchers in mid 1920s (Amaf, 2011).

The Bioplastics can be classified into four categories. They are the biodegradable plastics obtained from natural sources, polymers produced from microbial fermentation of agricultural products, polymers that are polymerized from oligomers or monomers and polymers derived from fossil fuel that can degrade biologically (Chan *et al*., 2020). Bioplastics can be produced from a variety of sources, including proteins, lipids and polysaccharides (Gonzalez-Gutierrez *et al*., 2010).

2.1 Algae as Bioplastic source

The Microalgal biomass is the potential source for producing bioplastics (Rahman and Miller, 2017). Amaf, 2011 suggested that Algae based plastics have been a recent trend in the era of bioplastics. Algae are photoautotrophic microorganisms that grow in faster rate with limited nutritional requirement. Algae are eukaryotic photosynthetic microorganisms. There are unicellular micro algae and multicellular macro algae are present in aquatic ecosystem. Algal biopolymers have advantages over plant based polymers. They are easily cultivable, non competitive with human food and can easily harvested throughout the year (Sreenikethanam and Bajhaiya, 2021). Studies suggested that production of bioplastic material from microalgae sources can be grouped into two main approaches. They are composites produced by blending microalgae biomass, bio- or petroleum-based polymers and additives (Önen *et al*., 2020).

The biodegradability of the plastic using algae is due to the presence of Poly Hydroxy Butyrate (PHB) in the microalgae cell (Das *et al*., 2018). The PHB is a polymer belonging to polyester class. The PHB has lot of industrial, medical and agricultural applications (Abdo and Ali, 2019). The presence of sodium acetate in culture medium enhances the PHB accumulation in algal cells. Monshupanee *et al*., 2016 suggested that presence of sodium acetate yield higher PHB accumulation in *Chlorogloea fritschii* Mitra (Peat and Whitton, 1967). Acetate is an efficient organic substrate for PHB accumulation (Singh and Sharma, 2012).

The different micro and macro algae have the potential to produce bioplastic film. The macro algae include Gelidium, Ulva etc after the enzymatic hydrolysis can be used for bioplastic production. The macro algae can form bioplastic film either directly or indirectly through the derivatives like agar, alginate, carrageenan (Lim *et al*., 2021). Sudhakar *et al*., 2021 studied the development and characterization of bioplastic from red sea weed. The polysaccharide in seaweed is uitilized for different industrial purposes. The higher tensile strength was resulted from seaweed based bioplastic using the plasticizer, Poly Ethylene Glycol (PEG).

The microalgae used for bioplastic studies include the genus *Chlorella*, *Spirulina*, *Calothrix* etc. Among the different microalgae *Chlorella* and *Spirulina* are the most commonly used algal species for bioplastic production. The *Chlorella* and *Spirulina* have different bioplastic properties (Önen *et al*., 2020). The higher protein content of *Spirulina* makes it more suitable for bioplastic production than *Chlorella*. The *S. platensis* has higher protein content and nutritional value among the different *Spirulina* species such as *S. maxima* and *S. fusiformis* (Hosseini *et al*., 2013).

2.2 Morphological features of *S. platensis* Gomont

S. platensis has a simple morphological structure with complex composition (Ravi *et al.*, 2010). It is a filamentous, spiral shaped Cyanobacteria. It belongs to the family Oscillatoriaceae. *Spirulina* is unicellular algae characterized by multicellular cylindrical trichomes. The cells are arranged in straight fashion which is enclosed by thin sheath (Anvar and Nowruzi, 2021).The major pigments of *S. platensis* are chlorophyll and phycocyanin. *S. platensis* contains all essential nutrients. The nutrients include 65% protein, 15% carbohydrates, 6% lipids, 0. 75% vitamins and 8% minerals. It is used as a healthy food in developing countries and sold in powder or tablet form (Ravi *et al.*, 2010).

2.3 Beneficial properties of *S. platensis* Gomont

Spirulina has anti viral properties. The bioactive compounds present in the cell can scavenges free radical. It has the inhibitory action against HIV 1, HSV 1, HSV 2, HCMV and influenza virus. The calcium molecules in *Spirulina* can reduce the viral multiplication about 50%. Some studies are reported that *Spirulina* extract can prevent cancerous cell. The methanolic extract of *Spirulina can* inhibit the breast cancer cells. The Beta carotene is a natural anti cancerous substance. The Beta carotene extracted from the algal cell inhibited the growth of DNA synthesis of sarcoma 180. *Spirulina* can reduce the chance of cataracts due to the presence of zeaxanthin and chlorophyll. It has lot of immunological applications. The *Spirulina* extact were used for chronic arsenic poisoning and keratosis (Anvar and Nowruzi, 2021).

2.4 Cultivation of *S. platensis* Gomont

Algae obtain all the nutrients and minerals from soil and water in natural habitats. All nutrients must be provided to algae in artificial culturing. All culture media contains source of Nitrogen, Phosphorous, Vitamins and Minerals. The most important nutrient for algal growth is Nitrogen and Phosphorous (Edmundson *et al.*, 2017). The highest production rates of algal cells were measured at Nitrogen and Phosphorous concentrations exceeding 25 and 2 mg litre (Mostert and Grobbelaar, 1987). Sharma *et al.*, 2017 studied the growth performance of algae in various nitrogen sources. It was revealed that nitrate is the suitable source for algal growth. The concentration of these elements is varying according to different media. The quality of the media can be determined through the growth performance of algae (Lananan *et al.*, 2013).

Dineshkumar *et al*., 2015 conducted studies related to the growth performance of *S. platensis* in different conditions in different culture media like zarrouk media, BG11 media, conway media, F/2 media and sea water. The BG11 media is the commonly used media for culturing green algae. From the studies he suggested that zarrouk media has higher influence on the massive growth of *S. platensis* compared to other culture media. The culture media become more basic as the culture attains older stage. Higher alkaline condition is necessary for the growth of *Spirulina*.

Soni *et al*., 2019 studied the effect of modified zarrouk media on the growth performance of *S. platensis*. Modified zarrouk medium was prepared using changing the nitrogen source. The concentration of algal cells was high when Urea is used as a nitrogen source instead of Pottasium nitrate. The nutritional components are the major factors which control the cell growth of *S. platensis*. The modified zarrouk media in presence of potassium nitrate shows higher cell growth (Chandrasekaran *et al*., 2016).

2.5 Starch based Bioplastic

Starch is a natural polymer material. Starch exists as granules in many plants such as corn, Cassava and Wheat (Song *et al*., 2011). The cassava starch contains two types of molecules such as linear and helical amylose and the branched amylopectin. The amylase content in the cassava starch is the main genetic trait that discriminate cassava varities (Chisenga *et al*., 2019). Mariappan reported that amylose content would improve the tensile properties of the bioplastics (Mariappan *et al*., 2019). Wahyuningtiyas and Suryanto, 2017 suggested that cassava starch shows greator potential for bioplastic production. They studied the effect of glycerol in bioplastic film on microbial degradation. From the studies, it is revealed that higher concentration of glycerol accelerate microbial degradation and extend the shelf life.

Bioplastic film made from different botanical source has different physiochemical and functional properties according to the starch characters. The starch concentration has also influence on the optical properties of bioplastic (Santana *et al*., 2018). Ghasemlo *et al*., 2013 investigated the performance of oil-coated starch and he suggested that the mechanical and water vapor permeability properties were improved for the use of packaging applications. The bioplastic film can be also made from banana peel using glycerol as a plasticizer to increase the flexibility.

2.6 Morphological properties of *Manihot esculenta* Crantz

Manihot esculenta Crantz belongs to Euphorbiaceae family. It has lot of regional names. It is commonly called as cassava, tapioca, yogo and manioc. It is a perennial plant with woody shrub. It has erect plant body. Cassava plant monoecious, contains separate male and female flowers on the same plant. Milky juice is present in the plant body. The palmate type of leaves with three to nine lobes is present. The tuberous roots of cassava are used as food. Cassava root contains chemical cyanogenic glucoside. In the presence of linamarase enzyme, glucoside becomes toxic cyanide (Fathima *et al.*, 2022).

2.7 Bioplastic studies on different bio sources

According to Önen *et al.*, 2020 *Chlorella* and *Spirulina* have high potential to produce bioplastic film. Additives like plasticizer, compatibilizer can improve the bioplastic properties. From the literature review he suggested that, there is more research is needed to make the bioplastic film available in market as a product. Amaf, 2011 compared the bioplastic prepared using *S. platensis* with commercial plastic. It was reported that biodegradability of bioplastic is high compared to commercial plastic due to the presence PHB in algal cells. The plasticizing and moldable property of both the plastics was good.

The maleic anhydride is used as compatibilizer in preparation of bioplastic film using *S. platensis*. It is useful to blend the bond between the microalgae and polymer. The bioplastic prepared using *S. platensis* with polyvinyl alcohol had higher tensile strength than commercial plastic bag. The presence of a compatibilizer smoothen the surface of bioplastic film (Ismail *et al.*, 2019). Maulida *et al.*, 2016 studied the bioplastic film using Cassava starch with Sorbitol as a plasticizer. He reported that highest tensile strength in bioplastic film was found in having 6% Micro-Crystalline Cellulose (MCC) and 20% Sorbitol. The plasticizer has a greater influence on the tensile strength of bioplastic. The tensile strength of the film was decreases with increases in the Sorbitol content. The results of moisture absorption showed that as the MCC increases, water uptake is decreases. The strong hydrogen bond in cellulose acts as a barrier to bond with water molecule.

Amaraweera *et al.*, 2021 reported that acid hydrolysis on starch can improves the structural and functional properties of starch. Acid hydrolysis improves the relative crystallinity of the films.

Abidin *et al.*, 2021 studied the bioplastic film using Cassava peel with different combinations of calcium carbonate and glycerol. The three different concentration of glycerol (20%, 30%, 40%) with constant amount of calcium carbonate is used for the analysis. The three different bioplastic prepared using different concentration of glycerol shows different mechanical properties and physical appearance. From tensile analysis, it is observed that 20% glycerol shows higher tensile strength. The degradation property of bioplastic was observed in highest glycerol concentration. Abidin suggested that bioplastic using 20% glycerol shows higher mechanical property hence it can replace synthetic polymer.

Fruit waste is also serves as a potential source for bioplastic production. The *Musa paradisica formatypica* peel is one among them to prepare bioplastic films. Sofiah *et al.*, 2019 studied the bioplastic production using *Musa paradisica formatypica.* The variations in the percentage of Sorbitol and glycerol affect the tensile strength and elongation percentage of bioplastic. From the studies, he concluded that 5 ml of sorbitol and 3 ml of glycerol concentration in bioplastic composite shows lowest tensile strength and the higher percent of elongation.

Vinodh, 2021 studied the bioplastic property of banana peel. It contain higher amount of starch. The fermentation of banana peel yield Poly Lactic Acid. Various additives such as vinegar, gluten etc. can increase the mechanical property of bioplastic. The usage of citric acid as a co-plasticizer can increase the tensile strength of bioplastic. Santana *et al.*, 2018 studied the starch based bioplastic film using Jackfruit with glycerol as a plasticizer. From his studies it is reported that Jack fruit can be used to generate bioplastic films with good mechanical properties. Mariappan Kadarkarainadar *et al.*, 2019 investigated the corn and starch based bioplastics. Different bioplastic films were generated using different combinations of starch with gelatin, citric acid and glycerol. Trujillo de Santiago *et al.*, 2015 determined the feasibility of producing bioplastic from Blue Maize and White Sorghum flours using thermoplasticization.

Additives such as plasticizers, compatibilizers, and various chemicals were used in bioplastic preparation to increase the quality of the final product. The additives increases the shelf life of bioplastics (Wahyuningtiyas and Suryanto, 2017). The plasticizers are organic molecules used as a blending material to increase flexibility and processability (Dianursanti *et al.*, 2018). The plasticizers can interact by means of hydrogen bonds with polymer matrix (Chapain *et al.*, 2021).

The plasticizers in the material films also affect the elastic modulus and tensile strength. The addition of plasticizers into Polysaccharide film is inversely proportional to tensile strength and elastic modulus (Badmus *et al*., 2015).

Glycerol ($C_3H_8O_3$) is the most commonly used plasticizer in bioplastic production using microalgae. The addition of glycerol as plasticizer in *Spirulina platensis* based bioplastic resulted in an improvement of elongation properties of the bioplastic (Dianursanti *et al*., 2018).The presence of glycerol allow the water to penetrate into the bioplastic film and assist in the biological or microbial processes (Wahyuningtiyas and Suryanto, 2017). The bioplastic film using glycerol as a plasticizer have higher film thickness than sorbitol.

Chapain *et al*., 2021 reported that bioplastic with glucose as a plasticizer showed the effective result in water uptake and soil decomposition test whereas the urea plasticized bioplastic showed relatively good tensile strength. The urea as a plasticizer exhibits a strong hydrophilicity due to its chemical structure, containing two amino groups and one carbonyl group. The addition of citric acid improves the shelf-life of the material and improves the mechanical properties (Mariappan *et al*., 2019). Compatibilizers are used to bind two polymers which consist of two parts; one part is compatible with one polymer and the other part is compatible with the other target polymer. It increases the mechanical strength of the heterogeneous biopolymers (Önen *et al*., 2020).

Tian *et al*., 2017 reported that Sorbitol as a plasticizer can influences the optical transparency, Crystallinity, Oxygen and water permeability, thermal stability, mechanical property and water retaining capability of polymer films. The Sorbitol can interact with Polyvinyl Alcohol and reduces the crystallinity of films. The water retaining capability of films is increases with increase in Sorbitol content. Gonzalez-Gutierrez *et al*., 2010 conducted studies to produce bioplastic film using oxidized starch with a plasticizer. The Sorbitol and Glycerol were used as a plasticizer. Sorbitol provides rigidity and brightness to the polymer film. The flexibility to the film was given by Glycerol. The dispersion of plasticizer on the oxidized starch improves the mechanical properties of the polymer film.

2.8 OBJECTIVES

- To analyse the pH dependent growth of *Spirulina platensis* in culture medium
- To analyse the growth response of *Spirulina* in modified culture medium
- To produce bioplastic using *Spirulina platensis*.
- To Produce bioplastic using *Manihot esculenta* using glycerol with different combination of acetic acid and calcium carbonate
- To produce bioplastic using *Manihot esculenta* with the addition of *Spirulina platensis*
- To compare the qualitative analysis (Moisture absorption, Biodegradability and Tensile strength, SEM analysis) of bioplastics.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS AND METHODS

This chapter deals with the materials and methods used for the analytical and experimental studies on the selected samples.

Biological Materials

The biological materials used for the present investigation are *Spirulina platensis* Gomont and *Manihot esculenta* Crantz. *Manihot esculenta* Crantz belongs to Euphorbiaceae family. *Spirulina platensis* Gomont is microscopic algae belongs to Oscillatoriaceae family. Algae collected was maintained in the Botany lab of St.Teresa's College, Ernakulam.

Glassware and chemicals

All the glasswares used for the analyses are Borosil glass. They were first washed with tap water with a detergent and again washed with distilled water. They were drained. Sudan Black and safranin are the two reagents used in the study. The chemicals used in the study are mainly for the preparation of Zarrouk's medium for algal culture (Table 1). The two major instruments used in the study are Scanning Electron Microscope (SEM) and Universal testing machine (Figure 2).

3.1. Collection of sample (*S. platensis* and *M. esculenta*)

The Cyanobacterium, *S. platensis* sample were collected from Central Marine Fisheries Research Institute (CMFRI), Kochi. The strain was maintained in Zarrouk's medium at 30 ± 2 ºC and pH 9 with continuous illumination using cool white fluorescent tubes (2500 Lux). Algae collected was maintained in the Botany lab of St.Teresa's College, Ernakulam. Microscopic observation where made to evaluate the morphological characteristics using Fluorescence microscope. The *M. esculenta* plant were collected from my home town vazhithala, Idukki District. The 250 gm of *Manihot* was collected.

3.2 Preparation of culture medium

3.2.1 Zarrouk's medium

Zarrouk media was prepared in double distilled water, used as culture medium. Loop full of *Spirulina* was inoculated in 250ml conical flasks with 150ml of media contents (Table 1) under sterile conditions. 10 components in the media (Table 1) were added to 1000ml double distilled water and mixed thoroughly. pH was adjusted to 8, 8.5, 9 and 9.5 using a pH meter and NaOH solution. Then poured four different pH solution of zarrouk's medium into 250ml four conical flasks. Mass culture of *Spirulina* was done in pH 10.The temperature was maintained at 30 ± 2 ºC. The illumination was provided with cool white fluorescent tubes (2500 Lux) under 12/12 hour light-dark cycles. Manual shaking of cultures was done in twice a day. The average number of algal strains was calculated using compound microscope.

3.2.2 Modified Zarrouk's medium

The *S. platensis* strains were also cultured in modified zarrouk medium in absence of A5 (Table 2) micronutrient and in presence of 2gm Sodium acetate. The temperature was maintained at 30 ± 2 ºC. The pH was adjusted to 8.5. The illumination was provided with cool white fluorescent tubes (2500 Lux) under 12/12 hour light-dark cycles. Manual shaking of cultures was done in twice a day.

3.3 Sudan Black Dye test

This test is used to detect the presence of Polyhydroxy butyrate in algal cells. The algal culture was heat fix on a glass slide and stained with 0.3% Sudan black stain. Then it is washed with 60% of ethanol. Counter stain the algal cells with 0.5% Safranin for five minutes and observed under Fluorescent microscope.

3.4 Preparation of bioplastic film using *Spirulina platensis*

Spirulina platensis was used as biological source for the preparation of bioplastic. *Spirulina* samples were filtered with a cheese cloth and dried in oven for 20 minutes. After complete drying *Spirulina* were used for bioplastic preparation. The bioplastic film was prepared using casting technique with Glycerol as a plasticizer. The 2.25gm of *S. platensis* was added with 2.25gm of Sorbitol, 2.25gm of Gelatin, 180ml of 1% Glycerol solution to 24ml distilled water. Then the solutions were stirred for 5 minutes before heated at 95ºC for 1hour.After the heat treatment, Poured the mixture into the aluminum plate and dried in oven at 60 ºC for 30 minutes. Then bioplastic was separated from the aluminum plate and subjected to qualitative analysis.

Table 1: Composition of Zarrouk’s medium

S. N	**Components**	**Amount g/L**
1	NaHCO3	16.8
2	NaNO3	2.5
3	NaCl	1.0
4	K2SO4	1.0
5	K2HPO4	0.5
6	MgSO4.7H2O	0.2
7	FeSO4.7H2O	0.01
8	CaCl2.2H2O	0.04
9	EDTA	0.08
10	A5 micronutrient	1
11	Distilled water	1000ml

Table 2: Composition of A5 micronutrient

S.N	Components	mg/ml
1	H3BO3	286
2	MnCl2.4H2O	250
3	ZnSO4.4H2O	22.2
4	Na2MoO4	7.9
5	CuSO4.5H2O	2.1
6	Distilled water	100

3.5 Preparation of bioplastic using *Manihot esculenta* (Cassava)

3.5.1 Extraction of starch from Cassava

250gm of Cassava tubers were washed, peeled, chopped into approximately 1 cm cubes and then ground in a high speed blender for 5 minutes. The pulp was suspended in 100ml distilled water for 10 minutes and filtered using a cheese cloth. The filtrate was allowed to stand for 1hr for the starch to settle and the top liquid was decanted and discarded. After decanting the top liquid, the sediment (starch) was dried in sunlight.

3.5.2 Preparation of bioplastic film

The bioplastic film was prepared using Glycerol as a plasticizer with different combinations of Calcium carbonate and Acetic acid (Fillers).5gm of Cassava starch was added to 70ml distilled water. Then the mixture was stirred up to 3 minutes. Acetic acid and calcium carbonate in different combination (Table 3**)** with 1ml of Glycerol was added to the mixture. Then the mixture was heated at 60ºC for 1 hour. Then the mixture was poured into aluminum plate and dried in oven at 60ºC. After complete drying the bioplastic was separated from the aluminum plate.

Table 3: Formulation of bioplastic using Cassava

S.N	**Cassava starch (gm)**	**Glycerol(ml)**	**Calcium carbonate(gm)**	**Acetic acid (ml)**
A	5	1	-	-
B	5	1	0.2	-
C	5	1	-	1
D	5	1	0.2	1

3.6 Preparation of bioplastic film using *Spirulina* with addition of Cassava

5gm of Cassava starch, 2.25gm of *S .platensis* were added to 70ml distilled water. Then the mixture was stirred up to 3 minutes.1ml of Glycerol and Gelatin was added to the mixture. Then the solution was stirred again for 5 minutes. The mixture was heated at 60ºC for 1 hour. Then the mixture was poured into aluminum plate and dried in oven at 60ºC. After complete drying the bioplastic was separated from the aluminum plate.

3.7 Qualitative analysis of bioplastic

There were four qualitative analysis of bioplastic was conducted.

- Moisture Absorption test
- Biodegradability test
- SEM analysis
- Tensile test

3.7.1 Moisture Absorption Test

Moisture absorption test was carried out according to ASTM D570 standard. Sample were cut into 1cm×3cm and dried in oven at 50ºC for 24 hours. The moisture absorption data of bioplastics was acquired by soaking the sample for 24 hours in water. After that, the bioplastic were dried with cloth and immediately weighed. Bioplastic moisture absorption capacity was calculated as in Equation 1.

$$\text{Moisture Content (\%)} = \frac{\text{(Post-Brake Weight)-(Initial Weight)}}{\text{Initial Weight}} \times 100 \qquad (1)$$

3.7.2 Soil Burial Degradation Test

Biodegradable behavior of bioplastics can be determined using soil burial degradation test. The extent of damage of bioplastics can be analyzed using degradation percentage. Bioplastic were cut into 1cm×3cm and weighed. Then the samples were buried into the ground at 8cm depth. The degradation rate of samples was calculated from the weight loss of the sample over time. The final mass of samples was measured after 7 days. The degradation of the test samples were calculated as in Equation 2 (Abidin *et al*., 2021).

$$\text{Degradation (\%)} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \times 100 \tag{2}$$

3.7.3 Observation of Morphological properties

The surface morphology of bioplastics was observed using Scanning Electron Microscope (SEM), FEI Inspect S50. Scanning Electron Microscopy (SEM) is a technique which is useful to observe the morphology of the bioplastic film in order to examine the interactions between different components in the composite It was used to observe the morphological characters of specimens. 10-nm- thick gold was coated on the samples before observation. It was done from Sophisticated Test and Instrumentation Centre (STIC) of Cochin University of Science and Technology (CUSAT).

3.7.4 Mechanical properties

Tensile strength of bioplastic is its ability to withstand under higher pressure without breaking (Vinodh, 2021).The tensile strength of samples was evaluated using CMT-10 Computer Control Universal Testing Machine (Figure 1) from the Department of Polymer Science and Rubber Technology in CUSAT. The sample width of narrow section was 10mm, thickness 0.3mm and gauge length 40mm.There are four specimens were tested for each sample.

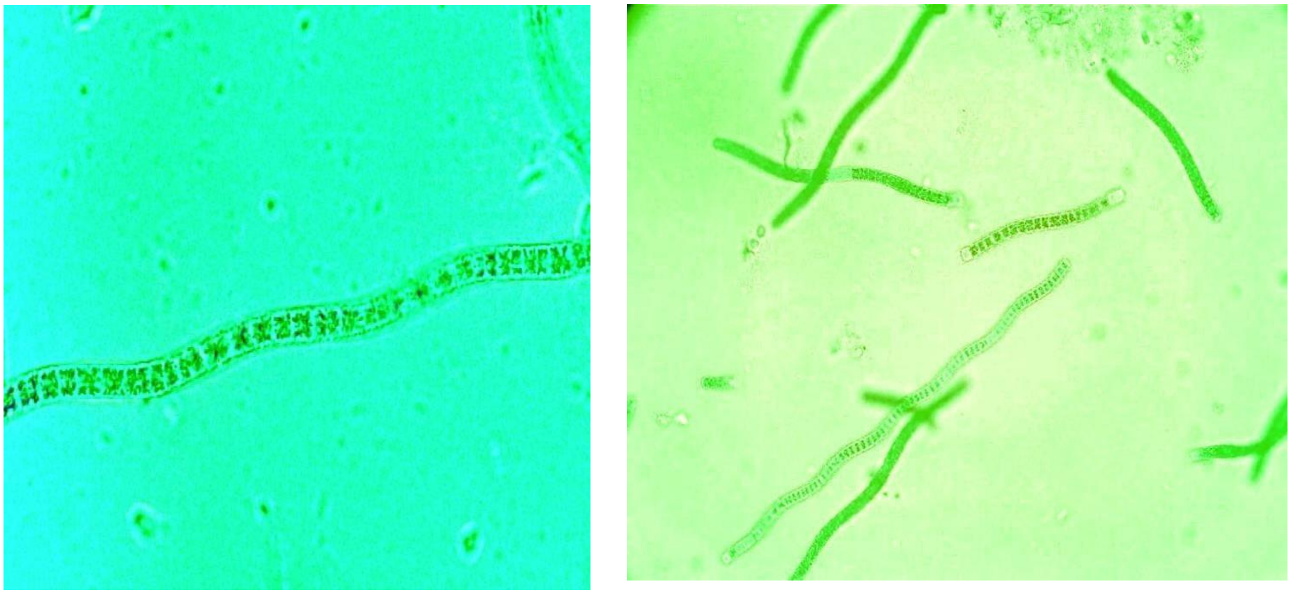
Figure 2: Image of Universal Testing Machine

CHAPTER 4

OBSERVATIONAND RESULTS

OBSERVATION AND RESULTS

Figure 2: Microscopic Evaluation of *Spirulina platensis*



Scientific classification of *S. platensis* Gomont

Domain : Bacteria

Phylum : Cyanobacteria

Class : Cyanophyceae

Order : Oscillatoriales

Family : Microcoleaceae

Genus : *Spirulina*

Species : *platensis*

S. platensis was observed as a spiral shaped filamentous unicellular algae.

4.1 Algae in culture medium

4.1.1 pH dependent growth of *S. platensis* in Zarrouk's medium

S. platensis was successfully cultured in zarrouk's medium. The periodical observations of culture medium after five days indicating the initiation of growth by fresh green colour under the pH 9.5. The higher growth rate of *S. platensis* was observed in pH 9.5. The average number of algal cells in pH 40.The samples kept under pH 8 show no visible changes. There were no algae was found in pH 8under the microscopic. It was observed that growth rate of *S. platensis* increases with increase in pH from 8 to 9.5 (Figure 5). The experiments revealed that highly alkaline condition is best suited for the growth of *S. platensis*.

4.1.2. Growth response of *S. platensis* in modified Zarrouk's medium

The modified Zarrouk's medium was prepared in absence of A5 micronutrient and in presence of Sodium acetate. The addition of sodium acetate to the culture medium was shown to be an effective to increase the algal growth. The average number of algal cells observed under the presence of Sodium acetate is 33. No algal growth was observed in the absence of A5 micronutrient. The results state that A5 micronutrient is essential for *S. platensis* growth. The presences of sodium acetate accelerate the algal growth. The average number of algal cells in pH 8.5 is 26. But in the same pH with 2gm Sodium acetate increases the average number of algal cells to 33. (Figure 6, 7, 8). The mass culture of *Spirulina* in pH was successful (Figure 9).

4.2 Evaluation of PHB in algal cells

Sudan Black test was conducted to evaluate the PHB in algal cells. Pink colour in algal cell was observed after sudan black test. The light pinkish colour indicates that *S. platensis* strain contains the presence of PHB in cell. (Figure 10).

4.3 Production of bioplastic

Bioplastic from *S. platensis* using glycerol as plasticizer was successfully produced. The bioplastic property of *S. platensis* is due to polyhydroxy butyrate content in algal cells. The bioplastic sheet was appeared in light green colour. The cholorophyll pigment in *S. platensis* gave the green colour to bioplastic sheet. Four different types of bioplastic were prepared using cassava (Figure 10). Cassava starch is a natural polymer. The starch was extracted from the cassava tuber after decantation process. 43.5gm of cassava starch was extracted from 250gm cassava tuber. Calcium carbonate was used as filler to strengthen the bioplastic films. Bioplastic films using cassava with addition of *S. platensis* was successfully produced (Figure 11, 12, 13, 14).

4.4 Qualitative analysis of bioplastic

4.4.1 Moisture absorption and biodegradability of bioplastics.

Qualitative analysis such as moisture absorption, biodegradability, SEM analysis and tensile test of bioplastic was conducted. Moisture absorption percentage of bioplastic was calculated according to the equation 1. The highest rate of moisture absorption was observed in bioplastic prepared using cassava with acetic acid. About 42.8% of water molecules are absorbed. The lowest rate was observed in bioplastic sheet prepared using cassava with calcium carbonate. It absorbed only 16.6%. The bioplastic prepared using *Spirulina* has lowest moisture absorption rate, 18.5%. The hydrophilic nature of acetic acid alone with glycine accelerate the water absorption rate. The presence of calcium carbonate reduces the water absorption rate. Calcium carbonate gives strength to bioplastic film. Biodegradation rate of bioplastics was calculated from first, second and third week using the equation 2. All bioplastics were degraded after three week. The bioplastic prepared using cassava with calcium carbonate showed lowest rate of degradation. The 58% of weight loss was observed in second week. The bioplastic prepared using cassava with acetic acid degraded in a faster rate. It was degraded after the first week. The moisture absorption and biodegradations studies showed that all the bioplastic prepared using cassava and *Spirulina* are biodegradable and can absorb water molecule. The rate of moisture absorption and rate of degradation varies according to the bio source and chemical used (Figure 15, 16, 17, 18).

4.4.2 SEM analysis

Surface microstructure of bioplastics were studied. Each of the bioplastic has different surface morphology. There are some granules, voids and cracks are observed. Granules are formed when the starch is not fully gelatinized. Cracks indicating the poor binding of components of bioplastic film. Voids indicates the poor interfacial adhesion between the components. Cracks are observed in bioplastic film prepared using calcium carbonate. It indicates that calcium carbonate is not fully gelatinized due to its hydrophobic nature. There were some bioplastics observed as smooth and uniform. Smooth and uniform structure structure of bioplastic were observed in bioplastic film prepared using acetic acid and glycerol. Voids are observed in bioplastic film prepared using *Spirulina* (Figure 19, 20).

4.4.3 Tensile test

Tensile strength of bioplastic was evaluated using Universal testing machine. Tensile strength of three bioplastic were studied. They are bioplastic prepared using Cassava, *Spirulina* and Cassava with addition of *Spirulina*. The highest tensile stress and strain were observed in bioplastic film prepared using cassava. Bioplastic prepared using *Spirulina* observed to have lowest tensile stress and strain. It was found that stress and strain are directly proportional to each other.

Table 4: Estimation of Algal cells in Zarrouk's medium

S.N	Different pH	Number of *S. platensis* strain (Mean ± SD)
1	8	0 ± 0
2	8.5	26 ± 2.7
3	9	29 ± 1.09
4	9.5	40 ± 3.53

Table 5: Estimation of Algal cells in Modified Zarrouk's medium

S.N	Different culture condition	Number of S. platensis Strain (Mean ± SD)
1	Absence of A5 micronutrient	0 ± 0
2	Presence of Sodium acetate	33 ± 1.14

Figure 4: *S. platensis* inoculated in Zarrouk's medium

Day 1

**Figure 5: *S. platensis* in Zarrouk's medium
15 days old**

Figure 7: *S. platensis* in

Modified Zarrouk's medium.

15 days old

Figure 8: Graph showing the specific growth rate of *S. platensis* in different culture conditions

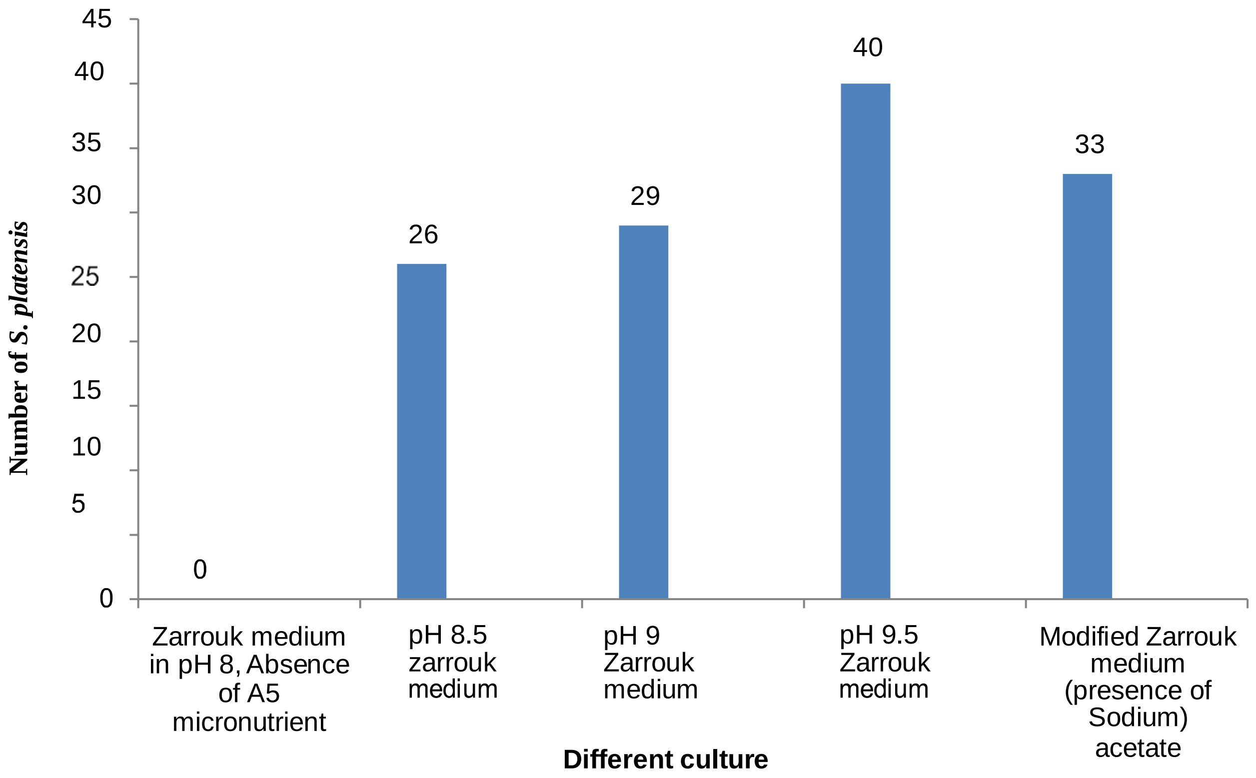


Figure 9: **A-*S. platensis* inoculated in pH10, Day 1.B-Mass culture of *S. platensis* after 15 days from inoculation.**

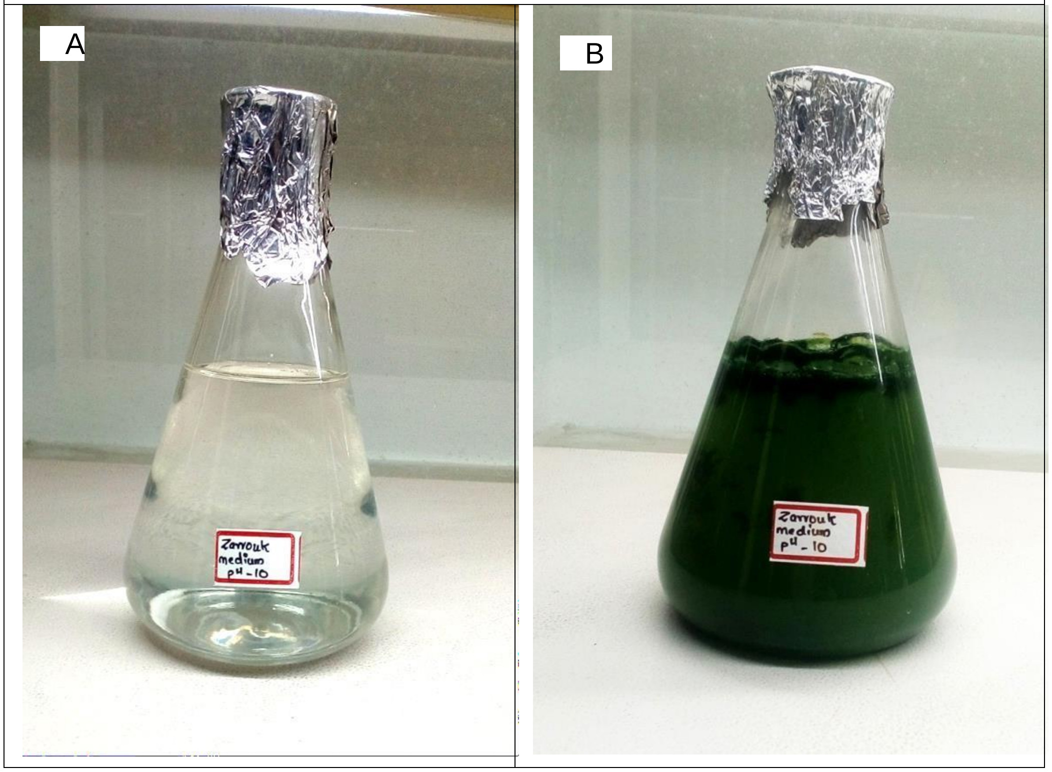


Figure 10: A- Evaluation of pure culture of *S. platensis.*
B-Evaluation of PHB in algal cells.

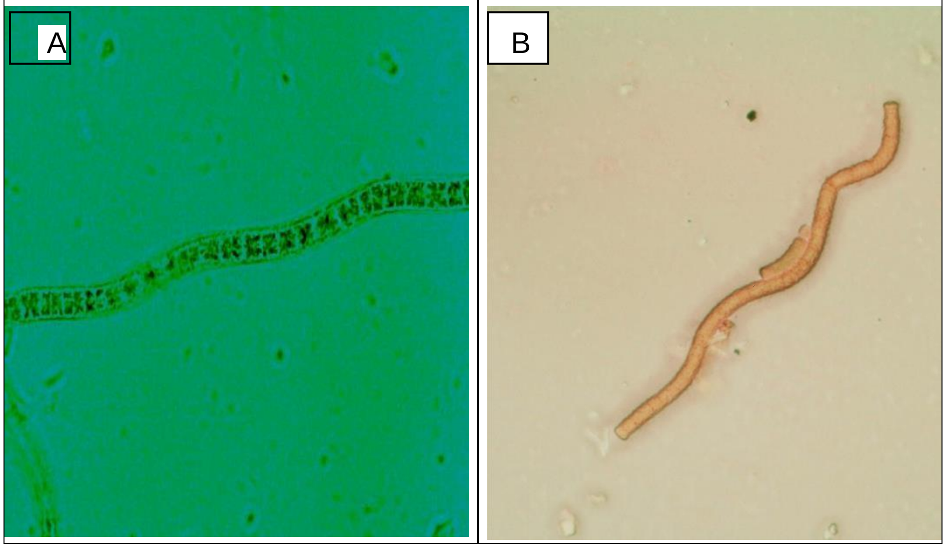
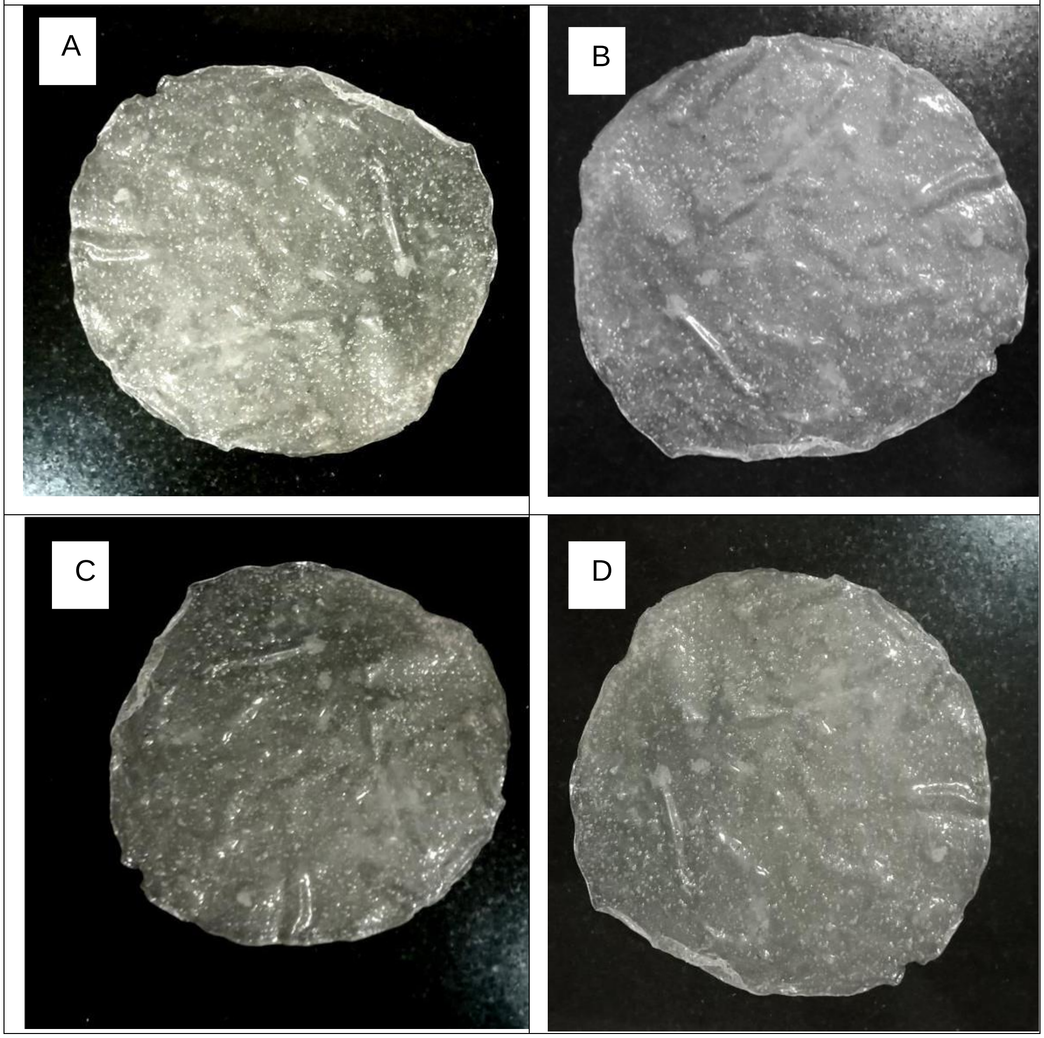


Figure 11: Image of bioplastic film produced from *S. platensis*

Figure 12: A - Cassava starch kept for settling. B- Cassava starch

Figure 13: Bioplastics produced from Cassava using Calcium carbonate and acetic acid



A-Cassava with Glycerol, B-Cassava with Glycerol and Calcium carbonate, C-Cassava with Glycerol and Acetic acid, D- Cassava with Glycerol, Calcium carbonate and Acetic acid

Figure 14: Image of bioplastic fim using Cassava with addition of *S*.

Figure 15: Moisture absorption test

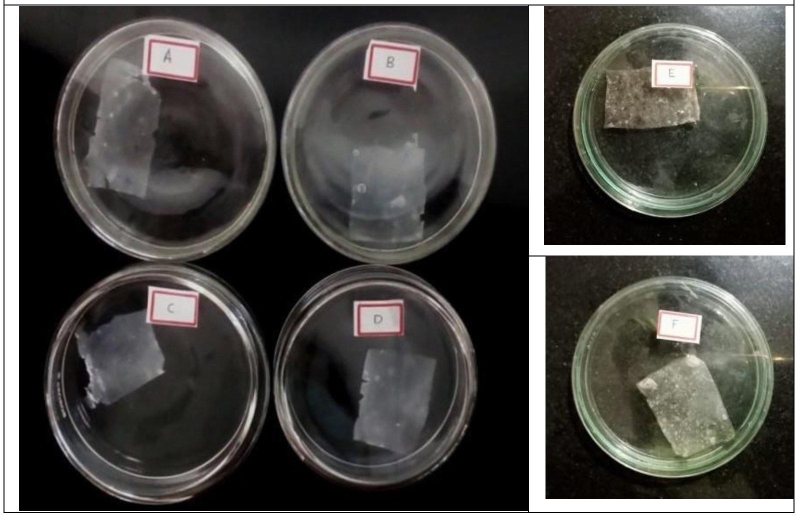


Table 6: Moisture absorption rate(%) of different bioplastics

SAMPLE	**MOISTURE ABSORPTION(%) Mean ±S.D**
A	30·7 ± 0.2
B	16·6 ± 0.18
C	42.8 ± 0.22
D	21·7 ± 0.21
E	18·5 ± 0.23
F	21.0 ± 0.16

Figure 16: Graph showing the moisture absorption rate of bioplastics

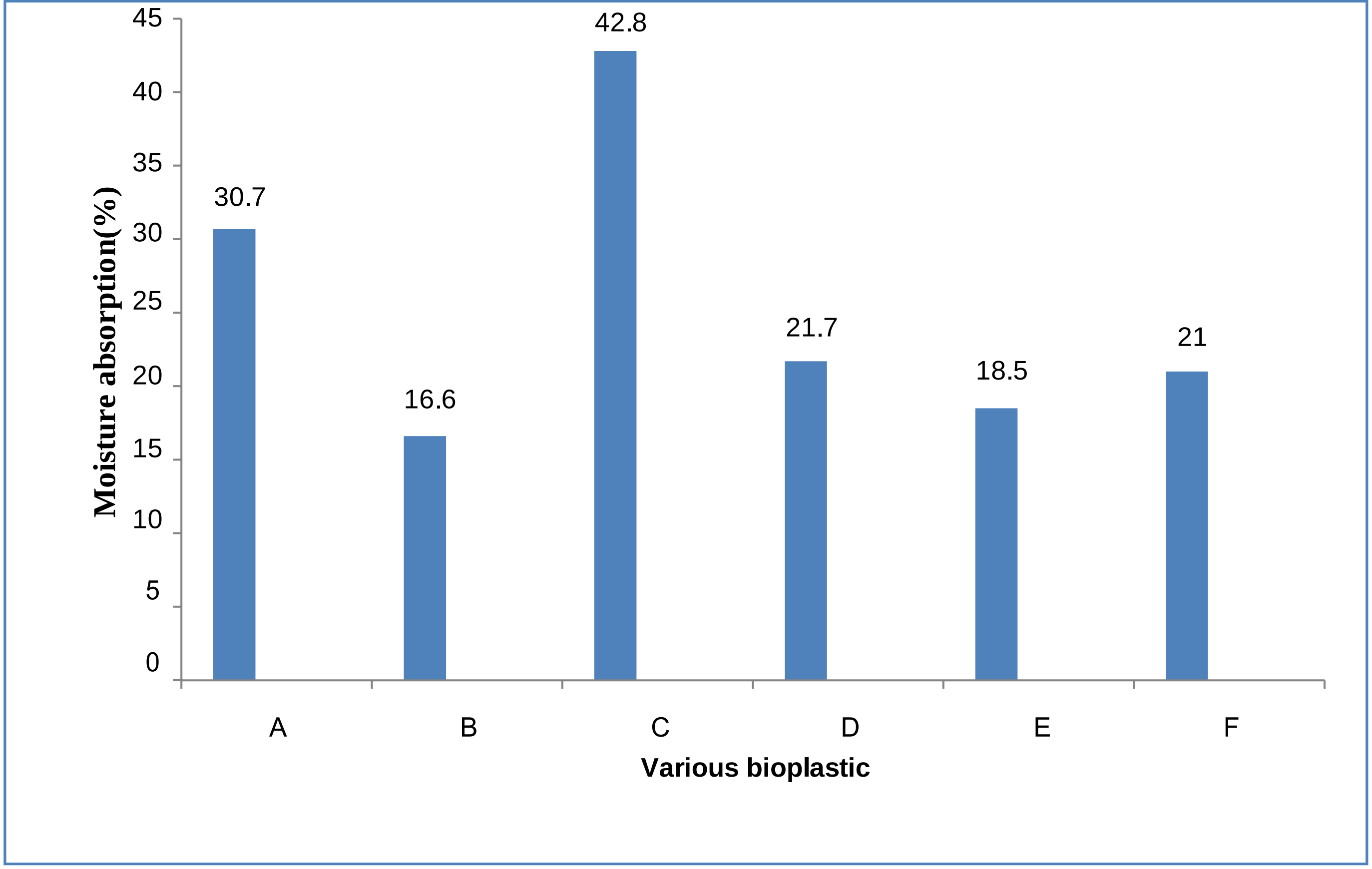


Figure 17: Bioplastic film buried in the soil

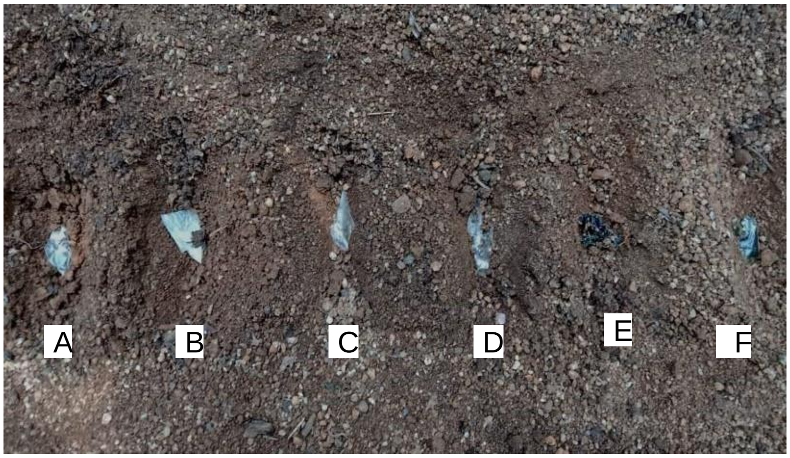
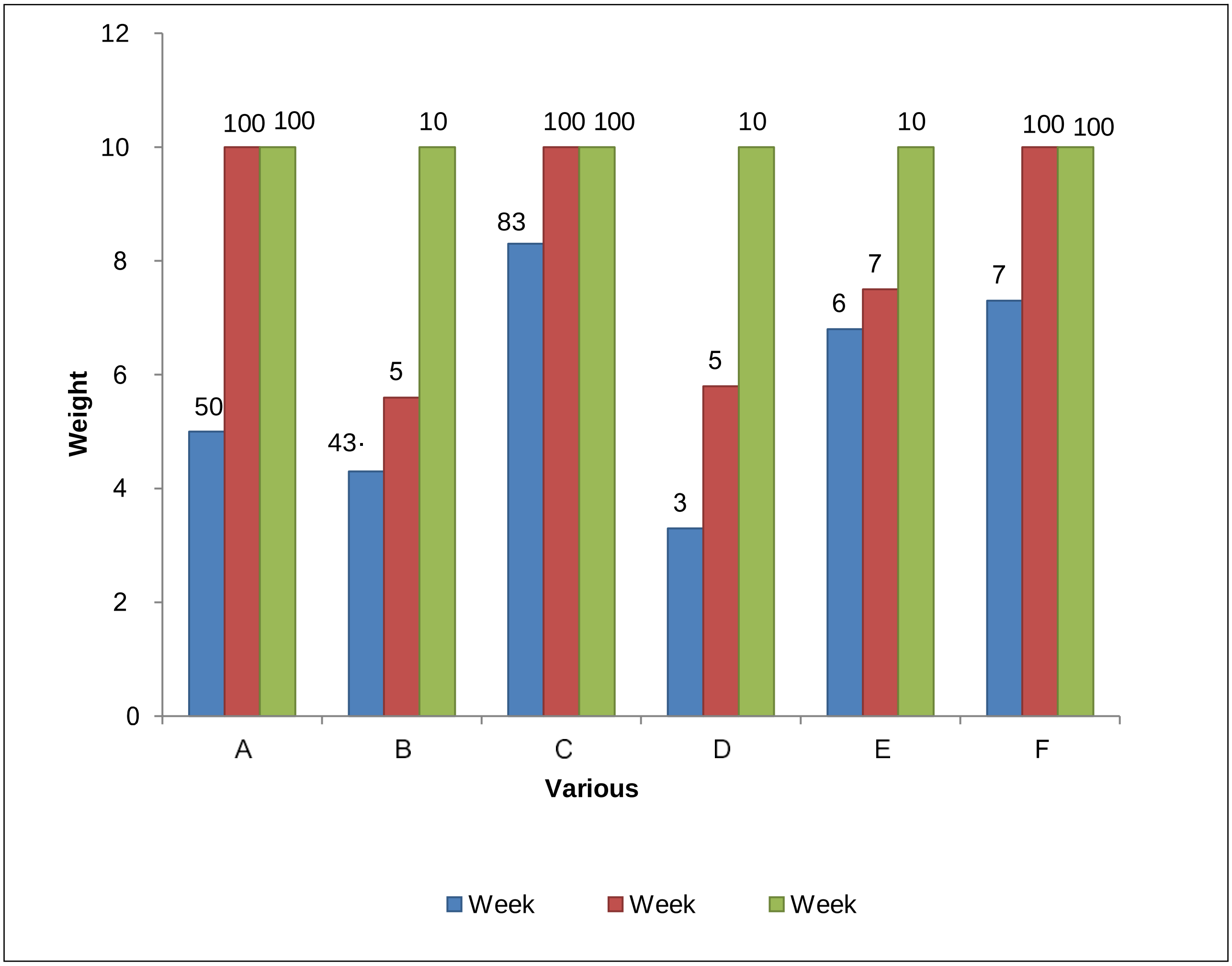


Table 7. Biodegradation rate of bioplastics

SAMPLE	**WEIGHT LOSS (%) mean ± S.D**		
	First week	**Second week**	**Third week**
A	0.6±0.2	0±0	0±0
B	0·9±0.1	0·7±0.11	0±0
C	0.2±0.18	0±0	0±0
D	0·8±0.2	0.5±0.17	0±0
E	0.5±0.19	0·4±0.15	0±0
F	0.5±0.1	0±0	0±0

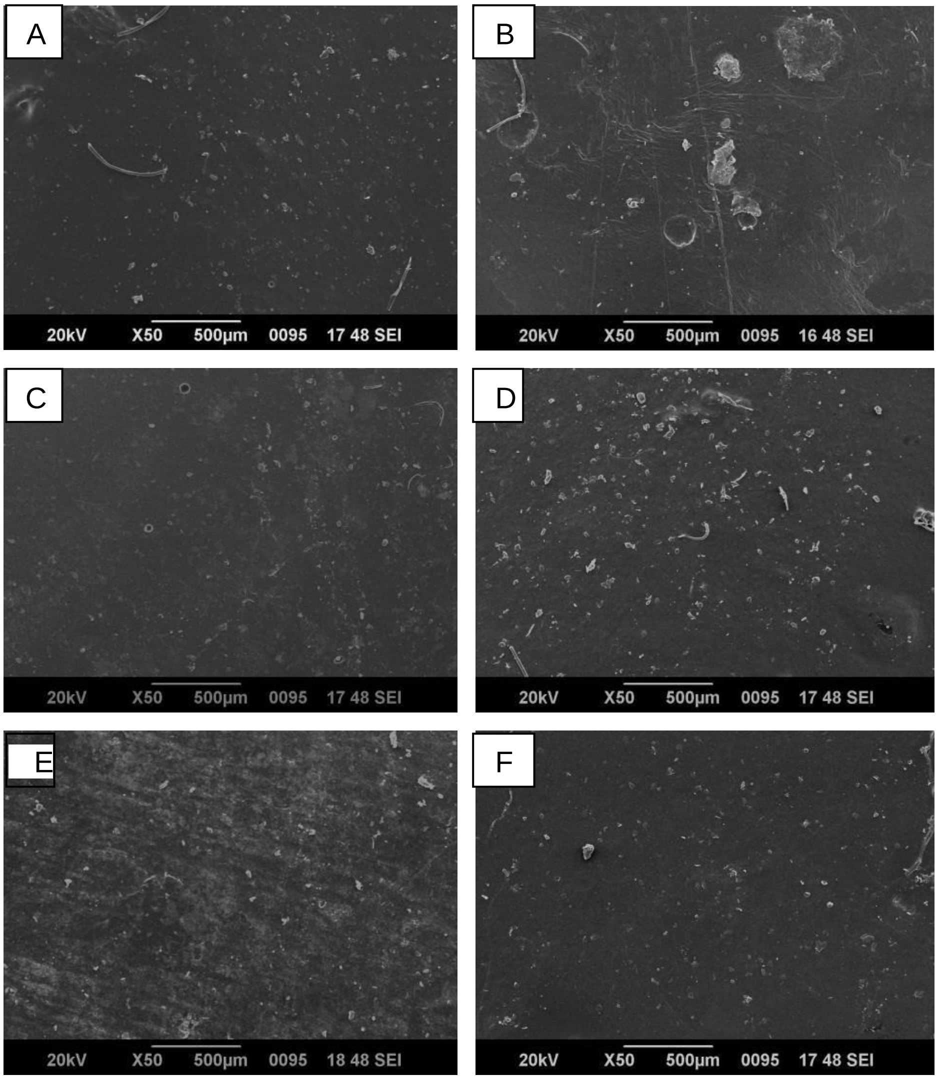
A-Cassava with Glycerol, B-Cassava with Glycerol and Calcium carbonate, C- Cassava with Glycerol and Acetic acid, D- Cassava with Glycerol, Calcium carbonate and Acetic acid, E- *Spirulina* alone, F- Cassava with addition of *Spirulina*

Figure 18: Biodegradation rate of bioplastics (%)



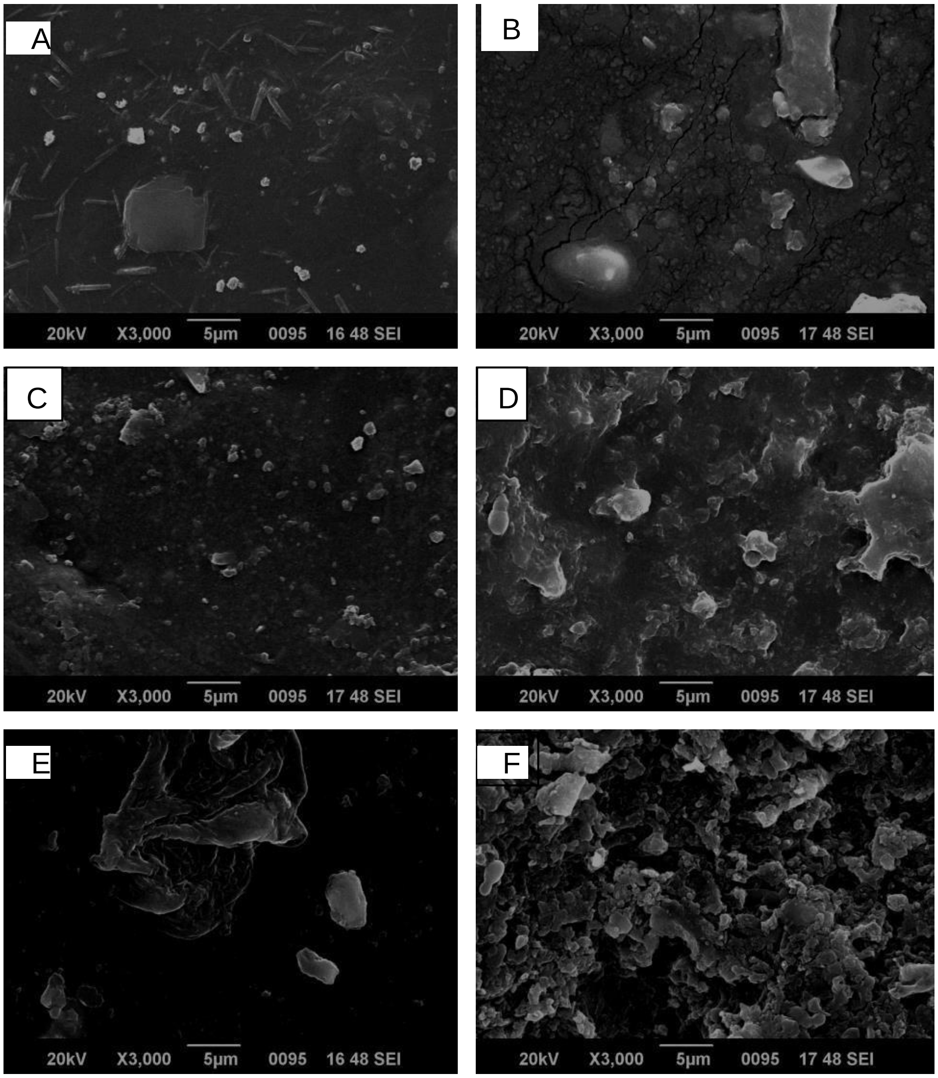
A-Cassava with Glycerol, B-Cassava with Glycerol and Calcium carbonate, C- Cassava with Glycerol and Acetic acid, D- Cassava with Glycerol, Calcium carbonate and Acetic acid, E- *Spirulina* alone, F- Cassava with addition of *Spirulina*

Figure 19: SEM image of bioplastics (50X)



A-Cassava with Glycerol, B-Cassava with Glycerol and Calcium carbonate, C- Cassava with Glycerol and Acetic acid, D- Cassava with Glycerol, Calcium carbonate and Acetic acid, E- *Spirulina* alone, F- Cassava with addition of *Spirulina*

Figure 20: SEM image of bioplastics (3000X)



A-Cassava with Glycerol, B-Cassava with Glycerol and Calcium carbonate, C- Cassava with Glycerol and Acetic acid, D- Cassava with Glycerol, Calcium carbonate and Acetic acid, E- *Spirulina* alone, F- Cassava with addition of *Spirulina*

Table 8: Tensile strength of bioplastic

SAMPLE	STRESS (MPa)	STRAIN (%)
	Mean ± S.D	Mean ± S.D
A	1·3±0.2	30.5±0.12
B	0·7±0.17	16.6±0.16
C	1.2±0.11	26·1±0.15

Figure 21: Graph showing tensile stress (MPa) of bioplastics

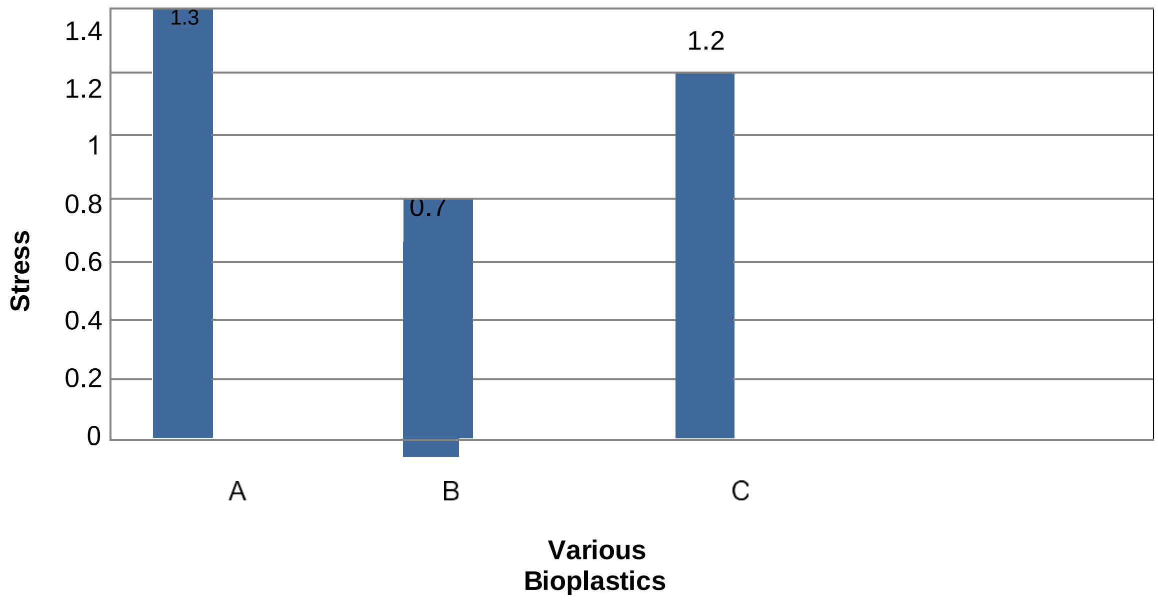
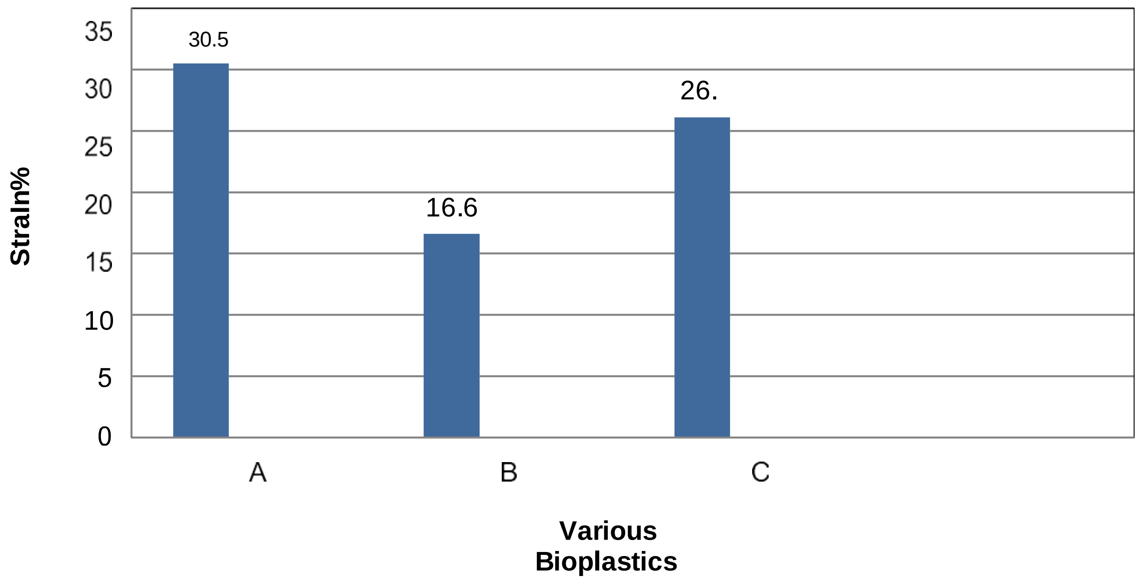


Figure 22: Graph showing tensile strain (%) of bioplastics



A- Bioplastic prepared using Cassava with calcium carbonate

B- Bioplastic prepared using *Spirulina*

C- Bioplastic prepared using Cassava with addition of *Spirulina*

CHAPTER 5

DISCUSSION

DISCUSSION

The present study is mainly focused on the on the bioplastic production from *Spirulina platensis* Gomont, *Manihot esculenta* Crantz and *Manihot esculenta* Crantz with addition of *Spirulina platensis* Gomont (Narancic *et al*., 2020). According to Onen *et al*., 2020 many different micro algae have the potential to produce bioplastic. *S. platensis* is selected due to its high amount of Polyhydroxybutyrate content. *S. platensis* is a spiral shaped cyanobacteria. It contains 50- 70% of protein content. They occur naturally in tropical and subtropical lakes. They have fast growing ability. Manihot esculenta Crantz belongs to Euphorbiaceae family. It has lot of regional names. It is a perennial plant with woody shrub.Starch present in cassava is a natural polymer and it exist in a granular form (Song *et al*., 2021). The bio sources such as *S. platensis* and *M. esculenta* (Cassava) are potential sources for bioplastic production. They are readily available sources.

Standardization of culture medium

Zarrouk medium is the commonly used medium for culturing *S. platensis*. *S. platensis* has been easily cultured in zarrouk's medium. It was the preliminary step during production of bioplastic from *S. platensis*. The pH depended growth of algae was evaluated. Algal growth was observed in four different pH such as 8, 8.5, 9 and 9.5. The dark greenish colour was observed in conical flask after one week from inoculation of *Spirulina*. Microscopic evaluation of algal cells was observed after 15 days from inoculation. There is no algal growth was observed under pH 8. The pH 9.5 was found to be the best suitable pH level for the massive growth of *S. platensis* in zarrouk's medium.High alkaline condition is the suitable environment for algal growth. The earlier report of Christwardana *et al*., 2011 based on the pH dependent growth of algae is confirmed.

Dineshkumar *et al*., 2015 conducted studies related to the growth performance of *S. platensis* in different conditions in different culture media like zarrouk media, BG11 media, conway media, F/2 media and sea water. The BG11 media is commonly used for culturing green algae. From the studies he suggested that zarrouk media has higher influence on the massive growth of *S. platensis* compared to other culture media. The growth response of *Spirulina* in a modified zarrouk's medium were also evaluated. Modified zarrouk's medium was prepared using sodium acetate. The growth of algae in absence of A5 micronutrient is also evaluated. Sodium acetate has a positive impact on the growth performance of algae. Without the presence A5 micronutrient algae didn't grow. It is reveled that A5 micronutrient is very essential for algal growth. The sodium acetate can accelerate the growth performance of *S. platensis*.

Bioplastic preparation

The bioplastics were prepared using two bio sources such as *S.platensis* and *M.esculenta*. *S. platensis* is a microscopic algae belongs to Oscillatoriaceae family. *M.esculenta* is commonly called as cassava. It belongs to Euphorbiaceae family. The Glycerol was used as a plasticizer for the bioplastic preparation. Glycerol is the most commonly used plasticizer. It improves the elongation properties of bioplastic. The plasticizers are used in bioplastic preparation to increase the flexibility and elasticity. The four different bioplastics were prepared from *M. esculenta* (Cassava). Cassava starch is a natural polymer. The preliminary step was the extraction of starch. The cassava starch was extracted from the cassava tuber after decantation process. From the 250 gram of cassava tuber 43.5 gm of starch was obtained. The calcium carbonate is also used as filler in bioplastic preparation. Acetic acid is used as a compatibilizer. Different combination of bioplastic using cassava were prepared using a constant amount of glycerol with different concentration of acetic acid and calcium carbonate.

Abidin *et al.*, 2021 studied the bioplastic film using cassava peel with different combinations of calcium carbonate and glycerol. Wahyuningtiyas and Suryanto, 2017 suggested that cassava starch shows greator potential for bioplastic production. They studied the effect of glycerol in bioplastic film on microbial degradation. From the studies, it is revealed that higher concentration of glycerol accelerate microbial degradation and extend the shelf life. Maulida *et al*., 2016 studied the bioplastic film using Cassava starch with Sorbitol as a plasticizer. He reported that highest tensile strength in bioplastic film was found in having 6% Micro- Crystalline Cellulose (MCC) and 20% Sorbitol. The plasticizer has a greater influence on the tensile strength of bioplastic. The tensile strength of the film was decreases with increases in the Sorbitol content. The results of moisture absorption showed that as the MCC increases, water uptake is decreases. The strong hydrogen bond in cellulose acts as a barrier to bond with water molecule. Bioplastic sheet using *Spirulina* is successfully produced. Gelatin is used as a compatibilizer. The bioplastic is also prepared using cassava with addition of *Spirulina*. (Abidin *et al*., 2021).

Qualitative analysis of bioplastic

The comparative study of qualitative analysis of bioplastics such as moisture absorption, biodegradability, tensile strength and SEM analysis test were conducted (Sudhakar *et al.*, 2021).The water absorption capacity of bioplastics were calculated by soaked the samples in water. The moisture absorption rate was calculated according to ASTM D570 standard. From the moisture absorption results, it was revealed that bioplastic prepared using cassava with calcium carbonate show least moisture absorption rate. The hydrophobic nature of calcium carbonate reduces the percentage of moisture absorption (Zeller *et al.*, 2013). The highest rate was found to be in cassava with glycerol and acetic acid. The increased water affinity of glycerol and acetic acid increases the moisture absorption percentage.

The extent of damage of bioplastics were analysed using soil burial degradation test. The several bacterial found in the soil such as *Pseudomonas* species, *Bacillus* species and *Moraxella* species has higher impact on the degradation of bioplastics. Biodegradation test gives rate of degradation percentage of bioplastic. The bioplastics samples were buried in the soil at 8 cm depth. The degradation rate of samples was calculated from the weight loss of sample over time. The final mass of samples was measured after 7 days . All the bioplastics are completely degraded after three weeks. The fastest rate of degradation was found to be in bioplastic prepared using cassava with glycerol and acetic acid. The hydrolysis reaction was takes place during the degradation process. The hydroxyl group of cassava starch initiates hydrolysis (Chapain *et al.*, 2021). The bioplastic prepared using calcium carbonate as filler degraded very slowly. The hydrophobic nature of calcium carbonate decreases the rate of degradation.

Scanning Electron Microscopy (SEM) is a technique which is useful to observe the morphology of the bioplastic film in order to examine the interactions between different components in the composite (Önen *et al.*, 2020). SEM analysis gives the information non gelatinized granules. SEM analysis was conducted to evaluate the surface morphology of bioplastic. It was done from Sophisticated Test and Instrumentation Centre (STIC) of Cochin University of Science and Technology (CUSAT). 10 nm thick gold was coated on the samples before observation. There are some granules, voids and cracks are observed. Granules are formed when the starch is not fully gelatinized. Cracks indicating the poor binding of components of bioplastic film. Voids indicates the poor interfacial adhesion between the components. Cracks are observed in bioplastic film prepared using calcium carbonate. It indicates that calcium carbonate is not fully gelatinized due to its hydrophobic nature. Surface features of bioplastic consist of granules (the remaining part of starch molecule). It indicates means that starch was not fully gelatinized during the formation process. Some voids were also visible on the fractured surface that had contributed to the low impact and tensile strength. Bioplastic prepared using Cassava with glycerol and acetic acid observed to be more uniform than others. The hydrophilic nature of glycerol and acetic acid makes the bioplastic film more uniform and smoother. Few cracks were observed in the bioplastic film using Cassava with calcium carbonate as filler. It indicates the poor binding of components (Santana *et al*., 2018).

Tensile strength of bioplastic is its ability to withstand under higher pressure without breaking (Vinodh, 2021). The tensile strength of three bioplastics were evaluated which include the bioplastic prepared using *Spirulina,* Cassava and Cassava with addition of Spirulina. The tensile strength of samples was evaluated using CMT-10 Computer Control Universal Testing Machine from the Department of Polymer Science and Rubber Technology in CUSAT. The sample width of narrow section was 10mm, thichness 0,3mm and guage length 40mm. There are four specimens were tested for each sample. The highest tensile strength was found in bioplastic prepared using Cassava with calcium carbonate (Mariappan *et al*., 2019). The maximum stress of bioplastic film using cassava was observed as 1.3Mpa with 30.5% strain. The lowest tensile strength was observed in bioplastic film prepared using *Spirulina*. It have 1.2 Mpa strain with 26.1% stress. The tensile strain were also increases as the stress increases. The highest tensile strength to bioplastic film was observed in film with lowest glycerol concentration (Santana *et al*., 2018).

CHAPTER 6

CONCLUSION

CONCLUSION

In this present study the production of bioplastic using *Spirulina platensis* Gomont, *Manihot esculenta* Crantz and *Manihot esculenta* Crantz with addition of *Spirulina platensis* Gomont have been successfully done. The comparative study of bioplastics was conducted by qualitative analysis such as moisture absorption test, biodegradability test, SEM analysis and tensile test. The plasticizer used to increase the elongation properties of bioplastic was Glycerol. The present study suggest that zarrouk's medium with pH 9·5 is best suited for the growth of *S. platensis*. The growth rate of *Spirulina* in zarrouk's medium increases with increase in pH. It was found that *S. platensis* in modified zarrouk's medium (presence of Sodium acetate) showed highest growth.

S. platensis was successfully cultured in zarrouk's medium. The pH dependent growth of *S. platensis* in Zarrouk's medium was studied. The algal growth was studied in four pH such as 8, 8.5, 9 and 9.5. The higher growth rate of *S. platensis* in pH 9.5 revealed that highly alkaline condition is best suited for the growth of *S. platensis*. The growth rate of *S. platensis* in modified Zarrouk's medium was studied. The *S. platensis* strains were cultured in modified zarrouk medium in absence of A5 micronutrient and in presence of Sodium acetate. The results states that A5 micronutrient is essential for *S. platensis* growth. The Sodium acetate was an effective nutrient for the massive growth of algae.

The moisture absorption test revealed that bioplastic using Cassava with acetic acid has high moisture absorption rate. About 42.8% of water molecules are absorbed. The lowest rate was observed in bioplastic sheet prepared using cassava with calcium carbonate. The bioplastic prepared using *Spirulina* has lowest moisture absorption rate, 18.5%. The soil biodegradation test indicates that biodegradability of Cassava starch based bioplastic with acetic acid is high. All the bioplastics were degraded after three weeks. The hydrophilic nature of acetic acid alone with glycerol accelerate the water absorption and biodegradability of bioplastic.

Surface microstructure of bioplastics were studied. Each of the bioplastic has different surface morphology. The surface microstructure of bioplastic film prepared using calcium carbonate indicates that calcium carbonate is not fully gelatinized due to its hydrophobic nature. Smooth and uniform structure structure of bioplastic were observed in bioplastic film prepared using acetic acid and glycerol. Voids are observed in bioplastic film prepared using *Spirulina*. The tensile test confirmed that bioplastic using Cassava with calcium carbonate as filler is stronger than other bioplastics with increased tensile strength. Bioplastic prepared using *Spirulina* showed lowest tensile stress.

REFERENCE

Abdo, S. M., & Ali, G. H. (2019). Analysis of polyhydroxybutrate and bioplastic production from microalgae. *Bulletin of the National Research Centre*, 43(1), 97.

Abidin, N. D. Z., Azhar, N. S., Sarip, M. N., Hamid, H. A., & Nasir, N. A. H. A. (2021). Production of bioplastic from cassava peel with different concentrations of glycerol and CaCO3 as filler. *AIPConference Proceedings*, 2332 (1),020004.

Afriani, Sari., Uju., Setyaningsih, Iriani. (2018). Chemical composition of *Spirulina platensis* which cultivated in photobioreactors with different photoperiods. *Pengolahan Hasil Perikanan Indonesia*, 21(3), 471.

Amara, A. A., & Steinbüchel, A. (2013). New Medium for Pharmaceutical Grade *Arthrospira.* *International Journal of Bacteriology*, 9(5), 188-1992.

Amaraweera, S. M., Gunathilake, C., Gunawardene, O. H. P., Fernando, N. M. L., Wanninayaka, D. B., Manamperi, A., Dassanayake, R. S., Rajapaksha, S. M., Gangoda, M., Fernando,

C. A. N., Kulatunga, A. K., & Manipura, A. (2021). Preparation and characterization of biodegradable cassava starch thin films for potential food packaging applications. *Cellulose*, 28(16), 10531–10548.

Amaf, U. (2011). Production of bioplastic using Spirulina platensis and comparison with commercial plastic. *Environmental science*, 4(3), 133–136.

Anvar, A., & Nowruzi, B. (2021). Bioactive Properties of *Spirulina*: A Review. *Microbial Bioactives*, 4(2), 134–142.

Badmus, A. A., Gauri, Samla., Ali, N. I., & Gomes, C. (2015). Mechanical Stability of Biobased Food Packaging Material. *Food Science and Quality Management,* 39 (1), 136-140.

Bezirhan, A.E., Ozsoy, H.D. (2014). Bio-plastics as a Green Material. International Conference on Green Infrastructure and Sustainable Socities /Cities, 14 (3), 66-70.

Bezirhan, E., & Bilgen, H. D. (2015). A Review: Investigation of Bioplastics. *Journal of Civil Engineering and Architecture,* 9 (8), 188–192.

Chan, J. X., Wong, J. F., Hassan, A., & Zakaria, Z. (2020). Bioplastics from agricultural waste. *Environmenal science*, 4 (7), 141–169.

Chandrasekaran, R., Ajeesh C.P, M., Sundaramoorthy, B., Shalini, M., Siva, R., Das, R., Fulzele, D., & Thiagarajan, K. (2016). Effect of Modified Zarrouk's Medium on Growth of Different Spirulina Strains. *Walailak Journal of Science and Technology,* 13 (8), 67–75.

Chandarana, J., & Chandra, S. (2021). Production of Bioplastics from Banana Peels. Bioactives, 24 (7), 88-90.

Chapain, K., Shah, S., Shrestha, B., Joshi, R., Raut, N., & Pandit, R. (2021). Effect of Plasticizers on the Physicochemical properties of Bioplastic Extracted from Banana Peels. *Journal of Institute of Science and Technology*, 26 (7), 61–66.

Christwardana, M., Hadiyanto, H., & Nur, M. M. A. (2011). *Spirulina platensis*: Potensinya Sebagai Bahan Pangan Fungsional. *Jurnal Aplikasi Teknologi Pangan,* 2 (8), 77-80.

Chisenga, S. M., Workneh, T. S., Bultosa, G., & Alimi, B. A. (2019). Progress in research and applications of cassava flour and starch: A review. *Journal of Food Science and Technology,* 56 (6), 2799.

Costa, J. A. V., Freitas, B. C. B., Rosa, G. M., Moraes, L., Morais, M. G., & Mitchell, B. G. (2019).

Operational and economic aspects of *Spirulina*-based biorefinery. *Bioresource Technology*, 292 (7), 121946.

Das, S. K., Sathish, A., & Stanley, J. (2018). Production Of Biofuel And Bioplastic From Chlorella Pyrenoidosa.*MaterialsToday:Proceedings*, 5(8), 16774–16781.

Demirbas, A. (2007). Biodegradable Plastics from Renewable Resources. *Energy Sources, Part A: Recovery, Utilization and EnvironmentalEffects*, 29 (5), 419– 424.

Dianursanti., Misri, Gozan.,and Citra, Noviasari. (2018). The effect of glycerol addition as plasticizerin *Spirulina platensis* basedbioplastic. *Phycology*, 67(8), 0304.

Dineshkumar, R., Narendran, R., & Sampathkumar, P. (2015). Cultivation of *Spirulina platensis* in different selective media. *Indian Journal of Geo Marine Sciences*, 45(12), 1749-1754.

Duranay, N. (2019). CO2 emission from combustion of lignite, waste plastics and biomass mixture pellets. *Chemical, Industry and Chemical Engineering,* 25(3*),* 239- 24.

Edmundson, S., Huesemann, M., Kruk, R., Lemmon, T., Billing, J., Schmidt, A., & Anderson, D. (2017). Phosphorus and nitrogen recycle following algal bio-crude production via continuoushydrothermalliquefaction. *AlgalResearch*, 26(8), 415– 421.

Fathima, A., Sanitha, M., Tripathi, L., & Kariuki, S. (2022). Cassava (Manihot esculenta) dual use for food and bioenergy: A review. *Food and Energy Security*, 23(7), 98-99.

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, 3(7), e1700782.

Ghasemlou, M., Aliheidari, N., Fahmi, R., Shojaee-Aliabadi, S., Keshavarz, B., Cran, M.J., Khaksar, R. (2013). Physical,mechanical and barrier properties of corn starch films incorporated with plant essential oils. *Polymers*, 98(1), 1117–1126.

Gonzalez-Gutierrez, J., Partal, P., Garcia-Morales, M., & Gallegos, C. (2010). Development of highly-transparent protein/starch-based bioplastics. *Bioresource Technology*,101 (6), 2007–2013.

Hosseini, seyede marzieh, Shahbazizadeh, S., Khosravi, K., & Mozafari, M. (2013). *Spirulina paltensis*:Food,Function. *Current Nutrition & FoodScience*, 9 (7), 189– 193.

Ismail, D., Noviasari, C., Windiani, L., & Gozan, M. (2019). Effect of compatibilizer addition in *Spirulina platensis* based bioplastic production.*Chemical science*, 2092(8), 030012.

Jangra, M., Shah, I., Batra, R., & Kumar, V. (2016). Poly-β-Hydroxy Butyrate production in bacteria employing ecofriendly and renewable agribyproducts. *The Ecoscan*, 10 (5), 81–84.

Jegan, S., Jegathese, S., & Farid, M. (2014). Review Article Microalgae as a Renewable Source of Energy: A Niche Opportunity.*International Journal of Sustainable Energy*, 5 (8) 95-99.

Kok Sheng, C., Senin, H., & Naimah, I. (2009). Structural and Mechanical Properties of Polyvinyl Alcohol (pva) Thin Film. *AIP Conference Proceedings*, 1136.

Krishnamurthy, A., & Amritkumar, P. (2019). Synthesis and characterization of eco-friendly bioplasticfromlowcostplantresources. *SNAppliedSciences*, 1(11), 1432.

Kumar, B., Pundir, A., Mehta, V., Singh, B., & Chauhan, R. (2020). A Review paper on plastic, its variety, current scenario and its waste management. *Plant Archives*, 20 (1), 53–56.

Lananan, F., Jusoh, A., Nora'aini, A., Lam, S. S., & Endut, A. (2013). Effect of Conway Medium and f/2 Medium on the growth of six genera of South China Sea Marine Microalgae. *Bioresource Technology*, 141(8), 60-67.

Lim, C., Yusoff, S., Ng, C. G., Lim, P. E., & Ching, Y. C. (2021). Bioplastic made from seaweed polysaccharides with green production methods. *Journal of Environmental Chemical Engineering*, 9(5), 105895.

Mariappan Kadarkarainadar, M., Jawaid, M., & Asim, M. (2019). Corn and Rice Starch-Based Bio-Plastics as Alternative Packaging Materials. *FIBE*, 7(1), 32.

Maulida, Siagian, M., & Tarigan, P. (2016). Production of Starch Based Bioplastic from Cassava Peel Reinforced with Microcrystalline Celllulose Avicel PH101 Using Sorbitol as Plasticizer. *Journal of Physics: Conference Series*, 710(1), 012012.

McAdam, B., Fournet, M. B., McDonald, P., & Mojicevic, M. (2020). Production of Polyhydroxybutyrate (PHB) and Factors Impacting Its Chemical and Mechanical Characteristics. *Polymers*, 12(12) 88-91.

Monshupanee, T., Nimdach, P., & Incharoensakdi, A. (2016). Two-stage (photoautotrophy and heterotrophy) cultivation enables efficient production of bioplastic poly-3-hydroxybutyrate in auto-sedimenting cyanobacterium. *Scientific Reports*, 6(1), 37121.

Mostert, E. S., & Grobbelaar, J. U. (1987). The influence of nitrogen and phosphorus on algal growth and quality in outdoor mass algal cultures. *Biomass*, 13(4), 219–233.

Narancic, T., Cerrone, F., Beagan, N., & O’Connor, K. E. (2020). Recent Advances in Bioplastics: Application and Biodegradation. *Polymers*, 12(4), 920.

Nishat, N., & Malik, A. (2013). Antimicrobial Bioplastics: Synthesis and Characterization of Thermally Stable Starch and Lysine-Based Polymeric Ligand and Its Transition Metals Incorporated Coordination Polymer. *ISRN InorganicChemistry*,23(8), e538157.

Önen Cinar, S., Chong, Z. K., Kucuker, M., Wieczorek, N., Cengiz, U., & Kuchta, K. (2020).

Bioplastic Production from Microalgae: A Review. *International Journal of Environmental Research and Public Health*, 17(5), 3842.

Peat, A., & Whitton, B. A. (1967). Environmental effects on the structure of the blue-green alga. *ArchivFürMikrobiologie*, 57(2), 155–180.

Rahman, A., & Miller, C. D. (2017). Chapter 6-Microalgae as a Source of Bioplastics. In R. P. Rastogi, D.Madamwar, and A. Pandey (Eds.), *Algal Green Chemistry*, 5*(4)* 121–138.

Ratih, P., Evi, A., & Se-Kwon, Kim. (2018). Photoprotective Substances Derived from Marine Algae. *Journal of Applied Phycology*, 29(2), 949–982.

Ravi, M., De, S. L., S, A., & Paul, S. (2010). The beneficial effects of Spirulina focusing on its immunomodulatory and antioxidant properties. *Nutrition and Dietary Supplements*, 2(1), 73–83.

Santana, R. F., Bonomo, R. C. F., Gandolfi, O. R. R., Rodrigues, L. B., Santos, L. S., dos Santos Pires, A. C., de Oliveira, C. P., da Costa Ilhéu Fontan, R., & Veloso, C. M. (2018). Characterization of starch-based bioplastics from jackfruit seed plasticized with glycerol. *Journal of Food Science and Technology*, *55*(1), 278–286.

Saranraj, P., (2012). Spirulina Cultivation: A Review. *International Journal of Pharmaceutical & Biological Archives*, 3(6), 1327-1341.

Sayeda M. Abdo & Gamila H. Ali. (2019). Analysis of polyhydroxybutrate and bioplastic production from microalgae. *Springer Nature*, 43(1), 99.

Selvamurugan, M., &Pramasivam, S. (2019). An Eco friendly alternative to petrochemical plastics. *Current World Environment*, 14(1), 49–59.

Sharma, J., Kumar, S., Sharma, P., Gupta, S., Toor, M., Malyan, S., & Bishnoi, N. (2017). Effect of Different Nitrogen Sources on Growth of Algal Consortia. *Annals of Agri Bio Research*, 22(1), 150–153.

Singh, B., &Sharma, D. (2012). Bioplastics-For Sustainable Development: A Review. *International Journal of Microbial Resource Technology*, 11(2), 11–23.

Sofiah, Yuniar, Aznury, M., & Melianti. (2019). Mechanical Properties of Bioplastics Product from Musa Paradisica Formatypica Concentrate with Plasticizer Variables. *Journal of Physics: Conference Series*, 1167, 012048.

Soni, R. A., Sudhakar, K., & Rana, R. S. (2019). Comparative study on the growth performance of Spirulina platensis on modifying culture media. *Energy Reports*, 5(5), 327–336.

Song, J., Kay, M., & Coles, R. (2011). Bioplastics. In *Food and Beverage Packaging Technology: Second Edition*, 3(8), 295–319.

Sreenikethanam, A., & Bajhaiya, A. (2021). Algae Based Bio-Plastics: Future of Green Economy. In *Biorefineries-Selected Processes* 9(4), 77-89.

Sudhakar, M. P., Magesh Peter, D., & Dharani, G. (2021). Studies on the development and characterization of bioplastic film from the red seaweed (Kappaphycus alvarezii). *Environmental Science and Pollution Research*, 28(26), 33899–33913.

Tian, H., Liu, D., Yao, Y., Ma, S., Zhang, X., & Xiang, A. (2017). Effect of Sorbitol Plasticizer on the Structure and Properties of Melt Processed Polyvinyl Alcohol Films. *Journal of Food Science*, 82(12), 2926–2932.

Trujillo de Santiago, G., de Gante, C., García-Lara, S., Verdolotti, L., Di Maio, E., & Iannace, S. (2015). Thermoplastic Processing of Blue Maize and White Sorghum Flours to Produce

Bioplastics. *Journal of Polymers and the Environment*, 23(1), 1–11.

Vinodh, J., Subasree, P., Mausheimi, G., & Sanju, R. (2021). Bioplastic from Banana Peel. *International Journal of Advance Research, Ideas and Innovations in Technology,* 5(2),89-99.

Wahyuningtiyas, N., & Suryanto, H. (2017). Analysis of Biodegradation of Bioplastics Made of Cassava Starch. *Chemical science*, 1 (7), 41–54.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E., & Brawley, S. H. (2017). Algae as nutritional and functional food sources: Revisiting our understanding. *Journal of Applied Phycology*, 29(2), 949–982.

Yaradoddi, J., Patil, V., Ganachari, Dr. S., Banapurmath, N., Hunashyal, A. M., & Shettar, A. (2016). Biodegradable plastic production from fruit waste material and its sustainable use for green. *International Journal of Pharmaceutical Research and Allied Science*, 9(6), 56–66.

Zeller, M. A., Hunt, R., & Sharma, S. (2013). Sustainable bioderived polymeric materials and thermoplastic blends made from floating aquatic macrophytes such as “duckweed.” *Journal of Applied Polymer Science*, 127(1), 375–386.

Zhong, X., Zhao, X., Qian, Y., & Zou, Y. (2018). Polyethylene plastic production process. *Insight - Material Science*, 1(3), 1-9.