

SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED BIOPLASTIC FILM USING HUMAN HAIR

Submitted in partial fulfilment of the requirement for the award of
Bachelor of Technology Degree in Chemical Engineering

by

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BONAFIDE CERTIFICATE

This is to certify that this project report is the bonafide work of S. GAYATHRI (39190004), N. MANASA (39190021) AND S.E. SUBASHINI (39190032) who carried out the project entitled "SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED BIOPLASTIC FILM USING HUMAN HAIR" under my supervision from November 2022 to April 2023

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We S. GAYATHRI (39190004), N. MANASA (39190021) AND S.E. SUBASHINI (39190032) hereby declare that the Project Report entitled **"SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED BIOPLASTIC FILM USING HUMAN HAIR"** done by me under the guidance of Dr.D. Prabhu M.S(By Research),Ph.D (Internal) and Dr.N.Nirmala M.Sc.,Ph.D (External) SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY, CHENNAI at is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in chemical engineering.

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ABSTRACT

Plastics are typically extremely strong polymers and one of the active ingredients that seriously harm the environment. The porosity of the soil is blocked by improper plastic bag disposal, which is problematic for groundwater resources. Plastics are not biodegradable, so leaving them in the environment as trash harms the environment permanently. The only way to address the growing threat of plastic waste to ecological sustainability is by creating biodegradable plastic, even from waste. Due to its stability against enzymatic degradation, keratin has gained interest and allowed for more predictable degradation profiles for tissue regeneration. Due to the presence of Keratin in human hair and it is highly microcrystalline, extremely strong, and resistant to both thermal as well as mechanical stress. The current study aims to extract keratin (protein) from human hair and use it to create a bioplastic film. Keratin is extracted using a sodium sulphide method, and bioplastic film is created by combining the extracted keratin solution with PVA and glycerol as a plasticizer. The keratin is confirmed by biuret test and FTIR. SEM, XRD, and FTIR will be used to characterise the prepared bioplastic film, and this paper will also discuss the material's mechanical, physical, and thermal properties.

Keywords: *Conventional plastic , Human hair , keratin , bioplastic film*

LIST OF CONTENT

CHAPTER NO	TITLE	PAGE NO
	ABSTRACT	v
	LIST OF CONTENT	vi
	LIST OF FIGURE	x
	LIST OF TABLE	xii
	LIST OF ABBREVIATION	xiii
1	INTRODUCTION	1
	1.1 PLASTICS	1
	1.1.1 An overview of plastic	1
	1.1.2 Plastic significance in modern culture	2
	1.1.3 Plastic production	5
	1.1.4 Impacts of conventional plastics	6
	1.2 BIOPLASTICS	8
	1.2.1 An overview of bioplastics	8
	1.2.2 Bioplastics as a closed loop approach	11
	1.2.3 Current state of bioplastics in India	12
	1.2.4 Biodegradation of bioplastics	13
	1.2.5 Sources of biomass	16
	1.3 KERATIN	18
	1.3.1 A Summary of keratin	18
	1.3.2 Human hair as a source of keratin extraction	20
2	LITERATURE SURVEY	22
3	AIM AND SCOPE	30
	3.1 AIM	30
	3.2 SCOPE	30
4	MATERIALS AND METHODS	31
	4.1 OVERALL PROCESS	31

4.2	SELECTION OF PROCESS	32
4.3	PRETREATMENT OF HUMAN HAIR	32
4.3.1	Weighing of human hair	33
4.3.2	Grinding of hair	33
4.3.3	Washing of hair	34
4.3.4	Delipidization and drying of human hair	34
4.4	EXTRACTION OF KERATIN FROM HUMAN HAIR	35
4.4.1	Dissolution of Human hair with sodium sulfide	35
4.4.2	Filtration process of dissolved hair	36
4.4.3	Centrifugation of filtrate	36
4.4.4	Dialysis of supernatant liquid	37
4.4.5	filtration and freeze drying of keratin sample	38
4.5	CHARACTERIZATION OF KERATIN SAMPLE	38
4.6	DERTERMINATION OF EXTRACTION YIELD	38
4.7	DETERMINATION OF MOISTURE CONTENT OF KERATIN SAMPLE	38
4.8	DETERMINATION OF COLOUR,TEXTURE AND WATER SOLUBILITY OF KERATIN	39
4.9	BIURET TEST	39
4.9.1	Biuret reagent preparation	39
4.9.2	Protein sample preparation	39
4.9.3	Procedure for biuret test	40
4.9.4	Observation of colour change	40
4.10	PRODUCTION OF BIOPLASTIC FILM	40
4.10.1	Methodology 1	40
4.10.2	Methodology 2	42
4.10.3	Methodology 3	44
5	RESULTS AND DISCUSSION	46
5.1	DELIPIDIZATION OF HUMAN HAIR	46

5.2 DISSOLUTION OF HUMAN HAIR	46
5.3 DIALYSIS OF SUPERNATANT LIQUID	46
5.4 EXTRACTED KERATIN SAMPLE	47
5.5 YIELD% OF EXTRACTED KERATIN SAMPLE	47
5.5.1 Effect Of Concentration And Time On Yield% Graph	48
5.6 SOLUBILIZATION OF HUMAN HAIR	49
5.7 KERATIN CONFIRMATION TEST	49
5.7.1 Confirmation of protein using Biuret test	49
5.7.2 Characterization of keratin sample	50
5.8 DETERMINATION OF MOISTURE CONTENT OF EXTRACTED KERATIN	51
5.9 COLOUR,TEXTURE AND WATER SOLUBILITY	52
5.10 BIOPLASTIC FILM TRIALS	53
5.10.1 Trial Run Pictures Of Methodology 1	53
5.10.2 Trial Run Pictures Of Methodology 2	53
5.10.3 Trial Run Pictures Of Methodology 3	54
5.11 FINAL OPTIMIZED METHOD FOR BIOPLASTIC PRODUCTION	55
5.11.1 Optimized bioplastic film	55
5.12 CHARACTERIZATION OF BIOPLASTIC FILM	56
5.12.1 Scanning Electron Microscope	56
5.12.2 X-Ray Diffraction	57
5.12.3 Fourier Transform Microscope	58
5.13 BIODEGRADABILITY TEST	60
5.14 ANTIMICROBIAL ACTIVITY OF BIOFILM	62
5.15 PROPERTIES OF BIOPLASTIC FILM	62
5.15.1 Thickness of film	62
5.15.2 Density of film	62
5.15.3 Moisture content of film	63

	5.15.4 Solubility of film	63
	5.15.5 Tensile strength of film	63
	5.15.6 Young's modulus of elasticity	63
6	SUMMARY AND CONCLUSION	64
	REFERENCES	65

LIST OF FIGURES

Figure no	Name	Page no
1.1	Flowchart for plastic production	5
1.2	Classification of bioplastics	10
1.3	Lifecycle of bioplastic	11
4.1	Overall process of keratin – based bioplastic film production	31
4.2	a) Experimental setup of weighing human hair	33
	b) Experimental setup of cutting human hair	33
4.3	Human hair washing with soap	34
4.4	a) Experimental setup of delipidization process	35
	b) Drying of treated hair	35
4.5	a) Dissolution of human hair in sodium sulfide	36
	b) Filtration of human hair	36
4.6	a) Centrifugation process	37
	b) Supernatant liquid	37
4.7	a) Liquid in cellulose tubular membrane	37
	b) Dialysis process	37
4.8	a) Filtration of Dialyzed sample	38
	b) Extracted keratin sample	38
4.9	Solution of keratin mixture	41
4.10	a) Polyvinyl alcohol Solution	43
	b) Hydrolysis of keratin solution	43
5.1	Sample of keratin powder	47
5.2	Graphical representation of effect of yield on concentration	48
5.3	Graphical representation of effect of yield on time	48
5.4	Changes in the sample after using biuret reagent	49

5.5	FTIR spectrum of produced keratin powder	50
5.6	Analysis of Moisture content	51
5.7	Solubility of keratin powder in water	52
5.8	Products obtained from methodology 1	53
5.9	Products obtained from methodology 2	54
5.10	Products obtained from methodology 3	54
5.11	Final bioplastic film	55
5.12	SEM analysis image of optimized film	56
5.13	XRD analysis image of optimized film	57
5.14	FTIR analysis image of optimized film	58
5.15	Biodegradability graph	60
5.16	Soil burial test	61

LIST OF TABLES

Table no	Name	Page no
4.1	Usage of chemicals	32
4.2	Concentration of sodium sulfide	36
4.3	No of trial runs and materials used in methodology 1	42
4.4	No of trial runs and materials used in methodology 2	44
4.5	No of trial runs and materials used in methodology 3	45
5.1	Components of keratin	46
5.2	Calculated yield%	47
5.3	Major functional groups identified in keratin	51
5.4	Colour, texture and water solubility	52
5.5	X-Ray diffractogram of extracted bioplastic film	58
5.6	Major functional group identified in bioplastic film	59
5.7	Calculation of weight loss% of bioplastic film	61
5.8	Total bacterial count of bioplastic film	62
5.9	Percentage of water solubility	63

LIST OF ABBREVIATIONS

BPA	- Bisphenol
EPA	- Environmental Protection Agency
PBT	- Persistent,Bioaccumulative,Toxic
UV	- Ultra Violet
RGF	- Regenerative Cellulose Film
PVA	- Polyvinyl Alcohol
RPM	- Rotation Per Minute
SBT	- Soil Burial Test
SEM	- Scanning Electron Microscope
FTIR	- Fourier Transform Infrared Spectroscopy
XRD	- X-Ray Diffraction
ASTM	- American Society For Testing Material
PCL	- Polycaprolactum
PLA	- Polylactic Acid

CHAPTER-1

INTRODUCTION

1.1 PLASTICS

1.1.1 An Overview of Plastic

Every year, roughly 330 million pounds of plastic are manufactured worldwide. Nearly 280 million metric tons of plastic were produced globally in 2012 only, according to estimates. Over 130 million metric tons of plastics throughout that total have been reused or dumped in landfills. Plastic will find a habitat in people's everyday life among 150 million metric tons that are left. Meanwhile, the remainder of the plastics percentage ends up as trash in water bodies or lying on the surface of the earth. As a result, the plastic trash poses a serious environmental threat to modern society since it contains several hazardous substances and, if improperly managed or cleaned, pollutes the Earth's soil, the atmosphere, and water bodies. Plastics are mostly used in the food packaging (40%), engineering (20%), and automobile (8%) sectors, as well as in the production of home products.

The overwhelming majority of industrialized plastics have no capacity for biodegradable and, as a result, cause environmental issues owing to a rise in accumulation of solid waste. Plastics are utilised in a broad variety of items that have swapped and displaced several other substances, like paper, timber, stone, leather goods, glass, metals, and ceramics, according to their easy nature of manufacturing, affordability, durability, and resistant to chemicals, weather, and lighting. Plastics are a prevalent material in contemporary society and are used in everything from spacecraft to stationary objects. Plastic is generally tough, expandable, waterproof, lightweight material, and extremely affordable to create. (Obebe SB & Adamu AA, 2020)

These facts are the appealing characteristics that encourage the excessive consumption of products made of polymer. As a replacement, plastic that is entirely formed of hydrocarbon products is particularly useful and degrades slowly. Plastic-

Pollution Association claims that the attractiveness of the plastic products we use on a daily basis has caused inescapable behavioural issues to emerge, which have resulted in overconsumption. At a given time, this behaviour seriously pollutes the surroundings. Prior research on the use of plastic debris in the production of construction has been documented.

Bags made of plastic, that are frequently used for packaging, carrying veggies and meat, among other things, have a major adverse effects on the surroundings. Because material bags can stay in the atmosphere over a maximum of one thousand years without decomposing, the number of plastic packs that are produced each year rises. Large amounts of plastic bags that are discarded or partially accumulated pollute the environment, water, and fresh air, damaging the biosphere and adversely impacting the organisms that live there.(Bandini et al., 2022)

Plastics cause a tremendous environmental impact. Those are resilient, lightweight, as well as adaptable, yet they degrade slowly and can remain in the surroundings for thousands of years. Above are some of the various ways that plastics harm the environment(Bandini et al., 2022):

1. Pollution: Plastic debris is a major pollutant in garbage dumps seas, and other sources of water. Plastics can require thousands of years to disintegrate, generating hazardous compounds and microplastics that damage biodiversity and ecosystems.
2. Habitat destruction: Plastic pollution may devastate animal habitats especially reefs and habitats for marine turtles.
3. Climate change: Plastic manufacture and disposal result in the release of greenhouse gases, which exacerbate global warming.
4. Impacts on human health: Plastics can include toxic chemicals like BPA (bisphenol A), and phthalates, which may enter water and food supplies as well as the well-being of humans.

1.1.2 Plastic's significance in modern culture

Polymers are more complicated than metals, it may categorise as natural or artificial 'macromolecules' comprised of lower repeating components. Organic polymers are synthesized in wildlife, plants, and both microscopic and macro-organisms. Polyunsaturated proteins, lipids, nucleic acid sequences, & rubber from plants are among examples. Cellulose, a highly prevalent organic compound on the planet, is a readily identified natural polymer. Artificial materials are polymers that are created by humans. The most significant among synthetic polymers include polyolefins. Plastics made from synthetic materials are prevalent in the environment, finding various uses in many industries due to their helpful features (mechanical and physiological properties) and low cost, contributing to the improvement of the ease and standard living in our current industrial civilization.

Polymers are not biodegradable they can be used in a variety of applications. Perhaps the most astounding characteristic of polymers produced from natural goods is the fact that such ecologically unstable substances should serve as the foundation for environmentally lasting goods used in industry. Rubber tyres represent the fewest ecologically stable of every polymer before manufacture, although vehicle tyres exist in the external environment over a while after usage. The following are the main applications of referred to as non-degradable thermoplastics(Thompson et al., 2009):

1. Surface coatings and paintings:Natural 'drying lubricants' made from unsaturated has been utilized to generations to preserve heavy metals through rusting and timber from bacterial activity. The most significant 'environmentally friendly' innovations that have developed were connected coverings and printing that used oligomeric acrylate polymers.
2. At workplace and Home:Plastics have emerged as the latest material option for devices performing at room temperature, including food mixers, hoover cleaners, hair dryers, tv consoles, desktops, word editors and various office equipment.
3. Transportation:Polymers are lighter than metals and ceramics. The current plastic dairy container weighs only a quarter of the capacity of a comparable glass bottle, which has a substantial impact on transportation costs. This

is widely held that milk delivered in recyclable glass cans is more environmentally friendly than single-trip containers made of plastic. Synthetic plastics are suited for this purpose due to their non-biodegradability. Polymers are also progressively switching traditional substances in machinery such as automobiles, aircraft, and watercraft, because of their long lifespans. All plastics having densities around one, but even the lightweight of regularly used metals, aluminium, is far heavier. It is projected that during the past ten years, 200-300 kilogram of conventional supplies in the normal vehicle would have been substituted by 100 kilograms of plastics, resulting a roughly 750 litre reduction in gasoline consumption throughout the usable lifespan of the vehicle. Furthermore, because the manufacturing of metals as well as glass requires more power than the generation of ordinary plastics, more polymers that could be utilised to replace alloys and glasses in automobiles, the less gasoline will be consumed in transportation. (Siracusa et al., 2008)

4. Agriculture: Plastics have affected rural areas due to their widespread used in farming, garbage disposal, and irrigation. Photo-degradable plastics might be ineffective because longevity of utilization is a significant consideration in these applications.
5. Packaging: Synthetic polymers have primarily been used as substitutes for standard materials, especially for packaging. Plastics are lightweight materials with excellent barrier characteristics against aquatic as well as water-borne microbes. They outperform glass in terms of hardness and robustness, resulting in less product loss while transportation. They safeguard the ecosystem not just from volatile goods, also from caustic or hazardous chemicals. Plastics made from petroleum-based products use significantly less labour and power than conventional substances (Thomas et al., 2012).
6. Public utilities: In the past few years, plastics have gained a more significant position in subsurface applications. In locations in which concrete or stainless steel were once employed, these include pipelines, ducting, and underneath chambers. Impermeable layers are now being used for a variety of new applications, such as the contamination of groundwater in storage tanks and

discharges in sanitary landfills, arrays and nets for soil stability, and underground electrical lines. Utilizing their capability to withstand biodegradation, synthetic materials are used in subsoil applications. An ever-growing component of utility delivery to commercial and home consumers is the underlying transportation of gas, electricity, and oil via pipeline(Meereboer et al., 2020).

7. Medicine and Biology:Polymers' biological stiffness and lightweight make them particularly appealing for future biomedical applications. Clinical applications are usual instances. Plastic prosthesis for replacement requires cutting-edge technology. However, in such application, the toughness with biocompatibility of every polymer under the severe circumstances to which products are exposed in use act as fundamental design criteria.

1.1.3 Plastic production

Oil and natural gas are just two of the numerous raw materials that are used in the production of plastics. Crude oil is first extracted, and after that it is processed to produce a variety of chemical compounds, such as ethylene, propylene, and benzene. Then, these chemical substances are utilised as a raw material for the manufacture of plastic. Figure 1.1 depicts the production of plastics in various ways.The three most popular processes for producing plastic are injection molding, blow molding, and extrusion(Kalita et al., 2021).

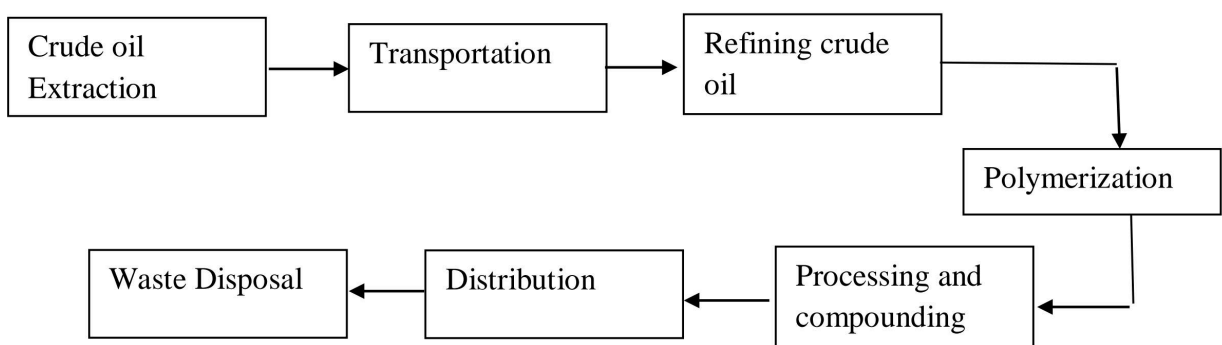


Figure 1.1 Flowchart for plastic production

1.1.4 Impacts of conventional plastics

- Effects of plastics on environment

Large amounts of plastic were found in dumps and outdoors. Plastic makes up about 10% of municipal trash discharge by weight, and it will be taken into consideration later. Newspaper reports of plastic trash on a few of the highest peaks demonstrate how leftover plastic can pollute a wide variety of natural surface, freshwater, and aquatic ecosystems. There are further some statistics on littering in cities. There are reports of small pieces of plastic inadvertently polluting soils as outcome of transmitting sewage effluent, of glass or plastic fragments affecting compost made from municipal garbage, and of plastics debris acted upon waterways, streams, and eventually the sea by rain and flash floods. However, more inquiry on the amounts and consequences of plastic litter in natural ecosystems, farmland, and freshwater is clearly needed. As a result, most of the proof provided here is naturally from aquatic environments(Austin et al., 2018).

- Effects of plastics on marine environment

Many plastics end up in the water, whether they disappear on ground or at marine. Plastics are transported far away from their primary source by wind power, rivers, sewage systems, and ocean waves. Plastics dumped in landfills can still reach the marine ecosystem if they have not been properly handled, especially if they come from windblown wastes or uncontrolled dumps near the shore. Plastic items collect on sandy shores and ocean floors, including in wetlands, mangrove forests, submarine trenches, frozen seas, and barrier reefs. Plastic trash on shores and coastlines comes from both local resources especially wind and waves propelled items that have probably migrated millions of kilometres. Single-use products such as cigarette packets, wrappers of food, plastic bottles, the caps and lids, disposable bags, utensils, and shakers take-away boxes, and foam-like polystyrene boxes are among the most encountered plastic objects in international ocean clean-ups. Several of those things are also frequently found on sandy beaches in New Zealand. Currently, 344 faunas have been documented to be entangled, which includes 100% of aquatic

turtles, 31% of whales, 67%- seals, 25% seagulls, 89 species aquarium fish, and 92 species of inverted vertebrates. Individuals that obtain plastics waste could be harmed by toxins connected to plastic products that are included during manufacturing or build up from polluted environmental matrices like soil or ocean. A minimum of 78% of the top pollutants designated by the US Environmental Protection Agency (EPA) have been established to be connected to polymer marine waste. Most of these compounds are Persistent, Bio accumulative, and even Toxic (PBT) (Thompson et al., 2009).

- Effects of plastics on human health

Plastics pose a risk to human wellness since they are used in healthcare processes and to safeguard our meals and drinks. Plastic vessels and bottles enable the distribution of safe-to-drink freshwater in areas where waterborne illness is a serious issue. Plastic wrapping prevents drinks and food from degradation due to microbial contamination. An increasing body of studies indicates that the continual accumulation of chemicals connected with plastics endangers our food's security and overall wellness. Microplastics were discovered in a variety of marine species, including fish as well as shellfish. It is further found in regular salt, well water, and up to 90% of H₂O that is packaged. Microplastics were recently found in the atmosphere and in household dust. From a daily basis, many people are likely inhaling in microplastics. Fortunately, it is unknown how much harm its exposure may cause humans or what happens when exposed to human beings.

- Effects of plastics on Animal

Many creatures, including reptilian species, mammals, vertebrates, and fish, could consume plastic waste when they mistake it as real prey and nutrition. The consequences can be hazardous with huge plastic pieces messing with an animal's ability to inhale, eat, or neutralize food, potentially leading in death. If plastics have been mistaken as meals and consumed, they may starve an organism, slow development, and impair the animal's ability to flee predators and breed. If microplastics are taken rather than food that can be digested, this results in

diminished feeding activity and even physical harm such lacerations and responses of inflammation(Dhar et al., 2018).

1.2 BIOPLASTICS

1.2.1 An Overview of Bioplastics

Bioplastics are plastics manufactured from renewable ingredients that include vegetable oils and fatty acids, corn starch, peas and beans starch and potato starch. It is seen as a greener choice to typical plastics derived from fossil fuels since they are both biodegradable and compostable, and they release greater greenhouse gases while processing. Bioplastics are receiving acceptance as a viable alternative to regular plastics in terms of environmental impact.

Bioplastics are an immersive changer in the sector of environmentally friendly substances. It is considered as a possible alternative to regular plastics due to growing concern about their environmental impact. In contrast to conventional plastics, that are generated from fossil fuels such as gasoline, bioplastics are created from renewable sources of biomass such as corn, sugarcane, and potato starch. One of the key advantages of bioplastics was their biodegradability(Austin et al., 2018).

This implies that bacteria can degrade them into natural chemicals like water, carbon dioxide, and biomass. As a result, they are compostable and have no impact to the build-up of plastic trash in garbage dumps and seas. Natural ingredients such as cellulose, starch and amino acids are commonly used to make biodegradable polymers. Enzymes, bacteria, and other microbes have the ability to degrade them.

The decreased carbon impact of bioplastics seems a further benefit. Traditional plastics are produced in large part using fossil fuels, that raises emissions of greenhouse gases and causes climate change. On the other hand, bioplastics are produced from sustainable sources resulting in a smaller carbon footprint. It can support sustainable development by lowering carbon emissions(Yin et al., 2013).

Bioplastics can be categorized in various ways depending on factors such as their sources, chemical compositions, production processes, and applications.

These definitions indicate some possible uses for bioplastics:

1. biodegradable and based on renewable resources;
2. although not being biodegradable, based on renewable resources;
3. based on fossil fuels and biodegradable

The two main types of bioplastics are biodegradable and non-biodegradable. Sustainable bioplastics are made to degrade under particular circumstances, like extreme heat or contact with particular microorganisms. The term "bio-based plastics," is also referred to as "non-biodegradable bioplastics," refers to plastics manufactured from renewable resources but which do not decompose. Figure 1.1 shows the classification of bioplastics. Both recycling and the production of new items can be done with these resources (Gironi & Piemonte, 2011).

- Advantages of bioplastic

Bioplastics have unique properties such as being beneficial to the environment, reusable and compostable, biodegradable, and energy efficient. The prospects for the use of biodegradable plastics are bright. Following list a few of the benefits of bioplastics:

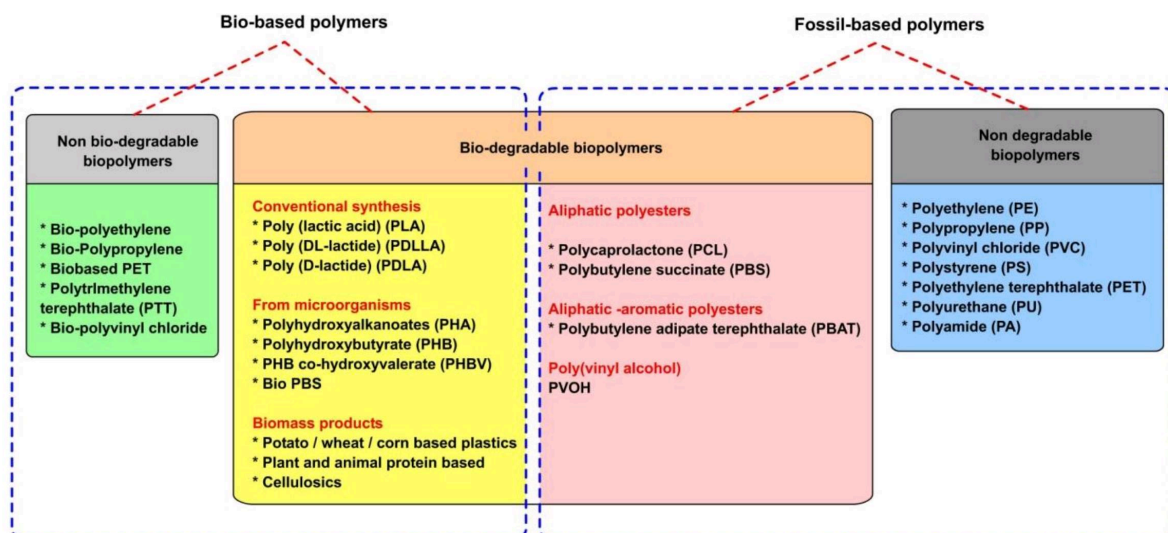


Fig.1.2; Classification of bioplastic

1. Renewable: Bioplastics can be created in an effective way because they are generated from renewable materials.
 2. Biodegradable: Some varieties of bioplastics are capable of decomposing harmlessly and biologically in the environment.
 3. Versatility: There are many applications for bioplastics, ranging from disposable items and packaging to car parts and surgical devices (Atiweh et al., 2021).
 4. Reduced carbon footprint: When bio-based plastics made from green energy sources, bioplastics could have a smaller carbon impact than conventional plastics.
 5. Reduced reliance on fossil fuels: Bioplastics can aid in reducing our reliance on finite, not sustainable fossil fuels.
- Disadvantages of Bioplastics

However, issues could arise while using bioplastics. The following are a few drawbacks of bioplastics:

1. Land usage: The crops required to manufacture bioplastics must be grown on land, which may impose pressure on habitats and agricultural practices.

2. Production concerns: Producing bioplastics may prove more expensive and labour-intensive than producing conventional plastics.
3. Misuse: Bioplastics can occasionally be confused for conventional plastics, that are result in them being placed in recycling systems where they are not intended, causing contamination, and lowering recycling rates.
4. Energy consumption: To make bioplastics, energy is needed, some of which may be derived from non-renewable resources that increase greenhouse gas emissions(Song et al., 2011).
5. Limited recycling option: There are not many options for recycling for bioplastics, even though some of them can be recycled. As a result, they might end up in incineration or landfills or in certain cases, they may be burned.

1.2.2 Bioplastics as a closed loop approach

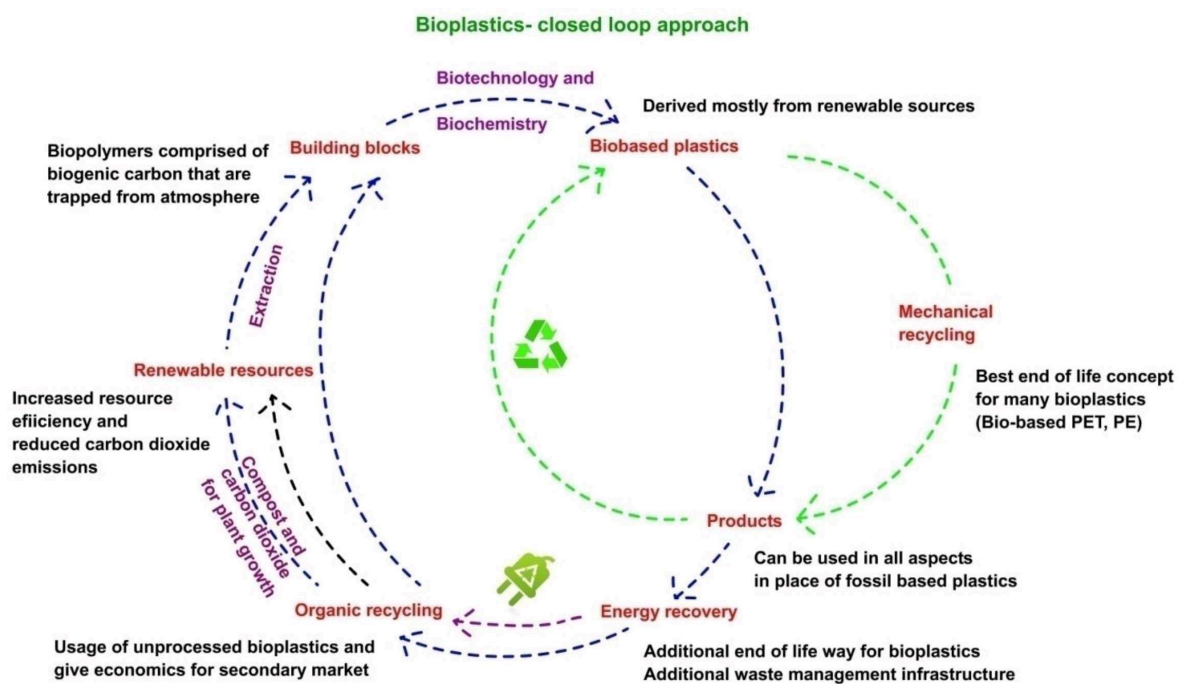


Fig.1.3; Life cycle of Bioplastic

Because they are made from renewable resources and can be composted, bioplastics can be regarded as a closed-loop strategy. Traditional plastics are not

biodegradable and are made from petroleum, an unrenovable resource, which causes pollution and harm to the environment.

On the contrary, sources of renewable energy that can be repeatedly cultivated and extracted include cellulose, sugarcane, and maize starch, which are used to make bioplastics. Additionally, bioplastics can be composted, where they decompose into organic material that can be applied as fertiliser naturally. The cycle is then continued by using the resulting compost to grow additional crops(Al-Salem et al., 2009).

Additionally, some bioplastics have the ability to be recycled, resulting in a closed-loop system where the materials can be used repeatedly before being composted at the end of their useful lives. As a result, fewer new resources are required, and less waste ends up in landfills and the oceans.

Figure 1.3 says bioplastics provide a more environmentally responsible and sustainable option to conventional plastics, offering a closed-loop system that encourages the effective use of natural resources and reduces waste(Grigore, 2017).

1.2.3 Current state of Bioplastics in India

Bioplastics have grown popularity in recent times because of their sustainability and biodegradability. The biodegradable plastics business in India is quickly expanding, with a yearly expansion rate around 30% estimated to hit a worth of USD 1.7 billion by 2025. This rise in consumption can be linked to people being more conscious of the adverse impacts of conventional plastics on the surroundings, as well as government aims to encourage sustainable practises. Several Indian companies have been producing bioplastics from sustainable resources including corn starch, sugarcane, and vegetable oil lately. These plant-based plastic are not solely biodegradable, but they also exhibit a reduced carbon impact than regular plastics. For example, Natur-Tec, makes bioplastics from a combination of corn and starch from potatoes. The organization's natural plastics are utilized in a variety of applications, includes packing, farming, and consumer products.

Another business, Bioplast India, produced a cornflour-based recyclable plastic. The items sold by the business have a life span of six months and may be used for a variety of things, including grocery bags, wrapping food, and temporary flatware. The business has also created a selection of recyclable plastic bags that may be used in place of conventional reusable bags(Sidek et al., 2019).

To encourage the usage of bio-based plastics in the nation, the Indian legislature has also launched several initiatives. Its announcement of a restriction on single-use plastics including drinking straws, utensils, and baggage in 2018 has spurred demand for sustainable substitutes. In order to promote the manufacturing of bio-based plastics in the nation, the government of India has also introduced a few programmes. In India, yet, there remains some issues required to be resolved despite the increasing prevalence of bioplastics. The price of biodegradable plastic is considerable compared to conventional plastics, which serves as one of the main obstacles. Due to their higher production costs, bioplastics are now less available for the consumers. Another issue that must be solved is the absence of construction for the disposal and reuse of bioplastics.

India's bioplastics business is expanding quickly, and it seems there is more opportunity for it to take a significant position in the worldwide bioplastics business. In the upcoming years, the need for reusable alternatives is anticipated to rise as an outcome of federal efforts and growing public awareness of the negative environmental consequences of conventional plastics. The expense and the absence of infrastructure for waste disposal and recycling are two issues that must yet be resolved(Rosenboom et al., 2022).

1.2.4 Biodegradation of bioplastics

The ability of bioplastics to break down much more quickly than conventional plastics is one of their primary benefits. Depending on the surroundings and the type of bioplastic, some bioplastics can biodegrade in a matter of months, whereas conventional plastics can take many generations to break down. Bioplastics may

degrade by means of enzymatic breakdown, hydrolysis, and oxidation, among other processes.

Bioplastics are broken down into simpler substances by enzymes through a process known as enzymatic degradation. Natural catalysts for chemical processes, or enzymes are materials formed by microorganisms like bacteria and fungi. Water and soil are two environments where enzymatic degradation can happen(Emadian et al., 2017).

Water breaks down bioplastics through a process known as hydrolysis. When exposed to both moisture and heat, bioplastics can undergo a process called hydrolysis in which the chains of polymer are broken down into simpler compounds. Compost and anaerobic digestion are two environments where hydrolysis can happen.

By being exposed to oxygen, bioplastics undergo a process known as oxidation. When bioplastics are exposed to air and UV light, they can oxidize, disintegrating into more basic compounds. Soil and water are two different environments where oxidation can happen.

The environmental advantages of bioplastics' biodegradation are numerous. Microplastics or dangerous chemicals are not left behind when bioplastics degrade. Water and carbon dioxide are natural byproducts of biodegradation that don't harm the environment(Russell et al., 2011).

The environmental effects of plastic contamination can be lessened by reducing the amount of bioplastic waste that ends up in landfills and oceans.

A promising area of study with the potential to lessen the environmental harm caused by plastic waste is the biological degradation of bioplastics. Bioplastics may breakdown much more quickly than conventional plastics in a variety of environments, such as soil, water, and compost on it. The potential of bioplastics to degrade in any environment and their emission of greenhouse gases while degrading are two issues that are connected to the biological degradation of bioplastics, though.

As research advances, it will be essential for reducing greenhouse gas emissions to develop bioplastics that can break down in all environments(Shavandi et al., 2017a).

- Factors affecting biodegradation

The kind of bioplastic is one of the most important factors affecting biological degradation of bio plastics. Each of the materials used to make bioplastics, including starch, cellulose, and PLA, has specific characteristics that have an impact on how quickly the material degrades. For instance, bioplastics made of starch degrade readily in compost but may not do so as well in soil or water. Contrarily, PLA may degrade in a variety of environments, such as compost, soil, and water, but they call for particular circumstances, like high temperatures and the presence of particular microorganisms.

1. Environment is the another element influencing the natural breakdown of bioplastics. Although biodegradation of bioplastics can occur in a variety of environments, including soil, water, and compost, the conditions needed for biological degradation can differ based on the environment. Bioplastics that breakdown in soil, for instance, might need different circumstances than those that degrade in water. The environment's moisture, temperature, and amount of oxygen can also have an impact on how quickly things degrade(Prasetyo et al., 2017a).
2. The breakdown of bioplastics is also influenced by their size and shape. Microorganisms can more easily access bioplastics that are minimal and have a lot of surface area, which can hasten the biodegradation of those materials. However, large or complex-shaped bioplastics might be more challenging for microbes to access, which could impede biodegradation(Folino et al., 2020).
3. Last but not least, the existence of additives in bioplastics may also influence how well they degrade. Some additives, like plasticizers and stabilizers, can stop microorganisms from rupturing the polymeric chains in bio plastics, thereby inhibiting the biodegradation of those materials. By encouraging oxidation and disintegration, other additives like pro-oxidant compounds and

photodegradable agents can hasten the breakdown of bioplastics(Harmaen et al., 2015).

1.2.5 Sources of biomass

- Cellulose

Cellulose is a highly abundant organic polymer on our planet and it acts as a straight homo-polymer of sucrose. In contrast to the structure in starch, a glucose repeated units are connected in this configuration. This permits the links to crystallize in a straight path as highly crystallized, compact, sub-micron the measurement micro-fibrils arranged along the cellulose fibre. Vegetable fiber, with certain exceptions such as cotton fibers, which is practically pure cellulose, existing as an organic bio-composite contains cellulose particles held together by hemicelluloses and the lignin substance found in plant cell walls to provide structural support. According to the degree of modification, there are several categories for direct cellulose use in wrapping, that range due to microfibrils, log pulp (small fibre), textile materials, or fibre optic technology, to comparatively untreated lignocellulosic biomass like wooden flour, drinking straws etc. Additionally, by altering the chemical composition of cellulosic derivatives, regenerated cellulose film (RGF) could be created(Camberos-Luna et al., 2016).

- Chitin

It is the second most widespread natural polymer on Earth, after cellulose. Chitosan originates from chitin. Chitosan actually a byproduct of the fishing industry that is produced through a breakdown process in an existence of alkali. It creates films without the use of chemicals and has superior mechanical and microbiological qualities against molds, microbes, and yeasts. This also has strong oxygen and dioxide flexibility, but the main disadvantage is the fact it is poorly soluble in neutral environments. The needed degree of breakdown to generate a dissolved product is 80-85%. It generates translucent films, which increases the product's value and storage life.

- Starch

Starch acts as a plentiful, inexpensive, and environmentally friendly form for organic polymer, making it a very desirable raw material for the production of bioplastics. The starchy substance is the carbohydrates copolymer of D-glucose that is found as granular particles in many crops, including corn, grain, rice, tapioca, & potatoes. varied sources of starch have varied ratios of different ingredients, molecular weights, varieties and levels of crystallinity, and granule sizes, which result in variations in characteristics. Because of its elevated temperature and brittleness, organic starch is unsuitable for use as a biodegradable plastic and needs to be reconfigured and plasticized while surrounded by of water using temperatures mechanical shear, when the addition of plasticizers such as glycerol and polyols. Compounded starch is used to bridge distinct polymer chains either with or without the use of bioplastics., produces an array of polymers known as starch complexes(Ezeoha, 2013).

- Proteins

Enzymes may break down proteins, which are organic chains of amines connected by amide bonds. Protein-based materials have been utilised as encapsulates, protective coatings, sticks, and cleansers since the 1930s. animal products and plant-derived proteins may be distinguished based on where they come from(Chilakamarry et al., 2021). Manufacturing food-safe containers is particularly appealing due to the nutritious benefits of proteins. Materials comprised of proteins coverings and films as well as edible film and coatings can be available with detailed explanations.

1. Animal base proteins: An important family of amino acids found in the extracellular layer of animal tissues is collagen. (bones, tendons, and skin). Collagen, which is decomposed by high-alkaline gives gelatine, it has been employed in food preparation and medication encapsulation. Plasticization, such as glycerin or mixing with different polymers, such as PVOH, might modify the physical and chemical characteristics(Sharma et al., 2018).

2. Plant based proteins: The beans (US) or soy (UK) has 38% amino and 18% oil, from other things. These were used to make polymers from soy. Soybean protein and oil are easily utilised to make sustainable highly rigid thermoset polymers, such as Soyad, which can substitute formaldehyde and urea glue in the fabrication of wood fibres board.

1.3 KERATIN

1.3.1 A Summary of Keratin

Keratin has increased in prominence over the past few years as a result of its remarkable biocompatibility and biodegradability. It has demonstrated promising outcomes in a variety of industries, including poultry, textiles, agriculture, personal care products and pharmaceuticals. Keratin acts as a multifunctional biopolymer which has been employed in the manufacturing of fibrous materials, and it may be converted into gel forms, films, nanoparticles, and microparticles with some necessary modifications. Its resistance to enzymatic breakdown and exceptional biocompatibility leading to applications for biomedicine and medical regeneration(Pourjavaheri et al., 2019a).

Keratin-based bioplastics are a form of bioplastic generated from a substance called keratin, the fibrous protein that occurs in hair, feathers, claws, and horns. It has been utilised in a variety of purposes, includes beauty products, healthcare and meals. Keratin-based biodegradable plastic have various advantages over standard petroleum-based plastics, which includes biodegradability, sustainability, and the ability for minimizing waste.

- Benefits Of Using Keratin Based Bioplastics

In this essay, we will look at the advantages of keratin-based bioplastics and why they can serve as a promising alternative to regular plastics.

1. Ecological Sustainability:Keratin-based bioplastics are regarded as environmentally friendly because they are created from a widely available

natural substance. Bioplastics are thought to be kinder to the environment than typical plastics derived from non-renewable resources that include oil. This is due to the fact that the manufacture of traditional plastics emits greenhouse gases, but the manufacture of bioplastics emits lower greenhouse gas emissions in general.

2. Renewable: Keratin is a renewable resource since it may be derived from a range of animal by-products, including hair and wings. As a result, keratin-based bioplastics are a more sustainable and greener alternative to petroleum-based plastics(Shavandi et al., 2017b).
3. Waste reduction: The usage of keratin-based bioplastics may help to reduce wastage by reusing otherwise discarded animal by-products. This has the potential to benefit both the surroundings and the local economy by reducing trash and providing a potential source of revenue for agriculture and other enterprises.
4. Strength and durability: Keratin is a tough and durable substance, making it a great choice for usage in a wide range of applications such as packaging and textiles(Pourjavaheri et al., 2019b; Ramakrishnan et al., 2018).
5. Flexibility: Depending on the intended usage, keratin-based bioplastics can be adjusted to fulfil certain specifications and needs, like flexibility or hardness.
6. Water resistance: Keratin-based bioplastics have been demonstrated to be extremely resistant to water, which makes them perfect for usage in applications such as wrapping food.
7. Low toxicity: Keratin is a natural material that is widely regarded as non-toxic, decreasing the possibility of harmful environmental or health effects linked with its use.
8. Potential for medical applications: Due to their strength and biocompatibility, keratin-based bioplastics have the ability to be used in medical products such as sutures.

9. Antibacterial characteristics: Some study suggests that keratin-based bioplastics may have antibacterial qualities, which could be valuable in medical applications or in decreasing food waste.
10. Challenges with sourcing: the keratin is a renewable resource, there are some challenges with finding and preparing it for usage in bioplastics. The grade and purity of the keratin can vary based on its source and processing methods utilized, which might have an effect on the qualities of the final bioplastic.
11. Cost: Due to the processing and purification required, the expense of generating keratin-based bioplastics may be higher than ordinary plastics. However, as manufacturing facilities and technology enhance the cost of these materials is projected to fall (Saharan & Sharma, 2015).
12. Limited availability: While keratin is renewable in nature, its accessibility and consistency can be affected by the source or processing methods used.
13. Potential for allergenicity: Keratin is a protein, and some people may be allergic to it. More research is required to determine the allergenic effects of keratin-based bioplastics.
14. Applications in packaging: Due to their rigidity and water resilience, keratin-based bioplastics have the ability to be utilised in a variety of packaging sectors, such as shipping supplies and food packing.
15. Textile applications: Due to their strength and durability, keratin-based bioplastics can be used for the manufacturing of textiles like as clothes and upholstery (Mohapatra, A., Prasad, S., Sharma, 2017).

1.3.2 Human hair as a source of keratin extraction

Keratin-based bioplastics are a viable alternative to standard petrochemical-based plastics since they are biodegradable, reusable, and non-toxic. Bioplastics can be made from a range of natural materials, including human hair. The main component of hair is keratin, a protein with fibrous structure that gives hair, nails, and skin structural strength. Keratin is a naturally occurring polymer with distinct features that

make it an excellent choice for bioplastic manufacturing. It is non-toxic, biodegradable, and has outstanding mechanical qualities such as high tensile strength, flexibility, and rigidity(Chilakamarry et al., 2021).

The easy availability and abundance of human hair as a raw material for keratin-based bioplastics is one of its key benefits. Hair is a natural resource that can be recycled and it is frequently generated by humans, which makes it a sustainable and environmentally benign option for bioplastic manufacture. Furthermore, because hair is frequently wasted as a consequence of cosmetics industry, reusing it as a raw material can help to decrease waste and pollution. Human hair has another benefit in that it is inexpensive and can be used to make bioplastics. In comparison to other keratin sources, like feathers or hooves, human hair is more affordable because it is readily available and easy to get. Hair is a versatile and readily accessible raw material because it may be procured in a variety of places, such as barber shops, salons, and even animal shelters(Maulida et al., 2016).

There are certain drawbacks to using human hair as a raw material, notwithstanding its long-term viability as a source for keratin-based bioplastics. One of the major issues is the variety in hair quality and composition, which might impact the qualities of the final bioplastic. For instance, chemically altered or damaged hair may result in a weaker or inferior bioplastic. Overall, human hair offers a promising source for keratin-based bioplastic films. It is a desirable alternative for producing sustainable bioplastics because of its availability, quantity, and affordability. To assure the creation of high-quality bioplastics, it is important to take carefully into account, the variations in hair content and quality as well as the difficulties in processing. Human hair-based bioplastics have the ability to replace conventional petrochemical-based plastics as a viable alternative with more research and growth(Thomas et al., 2012)

CHAPTER-2

LITERATURE SURVEY

- **Gomez-Martinez et al. (2011)** had published the journal in the topic of “Modeling of Pyrolysis and combustion of gluten–glycerol-based bioplastics”. Wheat gluten and gluten-based bioplastics' thermal degradation has been studied using non-isothermal thermo-gravimetric analysis in nitrogen and air atmospheres. Researchers prepared bioplastic from gluten and glycerol. They analyze the bioplastic by thermogravimetric evaluation, Thermal deterioration and the thermogravimetric model of gluten–glycerol-based bioplastics. Models based on pseudo components that correspond to the primary gluten isolate and bioplastic molecules, i.e., water, glycerol, starch, glutenin, and gliadin, have been used to simulate the thermal degradations of gluten and glycerol-gluten-based bioplastics. The simulation shows that the protein matrix and bioplastic moisture content both influence and impede glycerol volatilization.
- **M. Li et al. (2013)** extracted keratin from human hair using reducing agents and prepared bioplastics with high transparency and good mechanical properties. The researchers extracted keratin from human hair using a mixture of reducing agents, and used the extracted keratin to prepare bioplastics films. The bioplastics films exhibited high transparency and good mechanical properties. The results of plasticizer type and focus on the characteristics films made from bioplastic was also studied by the researchers.
- **J. Li et al. (2014)** created bioplastics from polyvinyl alcohol and human hair keratin, producing films with exceptional transparency and advantageous mechanical characteristics. In this work, keratin from human hair was isolated and polyvinyl alcohol, a biodegradable and water-soluble polymer, was used to create bioplastics. The solution casting technique was used by the researchers to create bioplastics, and they then examined the films' mechanical, thermal, and optical characteristics. The outcomes demonstrated that the bioplastics exhibited strong mechanical characteristics, for example, tensile strength,

modulus, and lengthening at break, as well as great transparency. The features were attributed by the researchers to interactions between the molecules of polyvinyl alcohol and keratin.

- **H. Shang et al. (2014)** synthesized bioplastics from keratin extracted from human hair and polyethylene glycol, resulting in films with high transparency and good mechanical properties. to create bioplastics using water-soluble and biocompatible polymer polyethylene glycol with human hair keratin that has been removed. The solution casting technique was used by the researchers to create bioplastics, and they then examined the films' mechanical, thermal, and optical characteristics. The outcomes demonstrated that the bioplastics exhibited strong mechanical characteristics, such as tensile strength, modulus, and elongation at break, as well as great transparency. The capacity of the plasticizer to improve the intermolecular connections between the keratin molecules is what the researchers said was responsible for the qualities.
- **T. M. Y. S. Tawakkal et al. (2015)** synthesized bioplastics from keratin extracted from human hair and polycaprolactone, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and polycaprolactone, a biodegradable and biocompatible polymer. The researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, Regarding the films' optical characteristics. The outcomes demonstrated that the bioplastics exhibited strong mechanical characteristics, such as tensile strength, modulus, and elongation at break, as well as great transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and polycaprolactone.
- **M. A. Rehman et al. (2015)** synthesized bioplastics from keratin extracted from human hair and polyethylene glycol, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and polyethylene glycol, a water-soluble and biocompatible polymer. The researchers prepared bioplastics using a solution casting method and tested the mechanical,

thermal, and the films' optical characteristics. The findings revealed that the bioplastics had high transparency and good mechanical attributes, including Tensile power, modulus, and lengthening at the break. The researchers attributed the properties to the plasticizer's ability to enhance the intermolecular interactions between the keratin molecules.

- **S. S. Jahan et al. (2015)** prepared bioplastics from keratin extracted from human hair and polyvinyl alcohol, resulting in films with high transparency and good mechanical properties. To prepare bioplastics from keratin extracted from human hair and polyvinyl alcohol, a biodegradable and water-soluble polymer. The researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, and optical qualities of the movies. According to the findings, the bioplastics possessed excellent mechanical qualities, including strong tensile strength, modulus, and elongation at break. The researchers attributed the properties to the intermolecular interactions between the keratin and polyvinyl alcohol.
- **N. H. Thanh et al. (2016)** synthesized bioplastics from keratin extracted from human hair and polycaprolactone, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and polycaprolactone, a biodegradable and biocompatible polymer. The solution casting technique was used by the researchers to create bioplastics, and they then examined the films' mechanical, thermal, and optical characteristics. The outcomes demonstrated that the bioplastics exhibited good mechanical attributes, such as modulus, elongation at break, and tensile strength, as well as great transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and polycaprolactone.
- **M. M. Yusuf et al. (2016)** prepared bioplastics from keratin extracted from human hair and glycerol, resulting in films with high transparency and good mechanical properties. To prepare bioplastics from keratin extracted from human hair and glycerol, a natural plasticizer. The researchers prepared bioplastics using a solution casting method and tested the mechanical,

thermal, and optical characteristics of the films. The outcomes indicated that the bioplastics had high transparency and good mechanical characteristics, including elastic modulus, modulus, as well as elongation at break. The researchers attributed the properties to the plasticizer's ability to enhance the intermolecular interactions between the keratin molecules.

- **M. A. Al-Ghouti et al. (2017)** investigated the extraction of keratin from human hair using various reducing agents and characterized the extracted keratin using FTIR, XRD, and SEM. The study found that sodium sulfite and sodium hydroxide were effective in extracting keratin from human hair, and the extracted keratin exhibited a high degree of purity.
- **K. R. Rajendran et al. (2017)** used different solvents and plasticizers to prepare bioplastic films from human hair keratin with high transparency and biodegradability. This study explored the use of different solvents and plasticizers to prepare bioplastic films from keratin extracted from human hair. The study found that the resulting bioplastic films had high transparency and biodegradability, making them promising candidates for eco-friendly packaging materials.
- **R. T. Pawar et al. (2017)** investigated the outcome of plasticizer type and focus on the characteristics of bioplastics made from keratin in human hair. The researchers synthesized bioplastics films from keratin extracted from human hair, and examined the effect of different plasticizers (glycerol, sorbitol, and polyethylene glycol) on the properties of the bioplastic's films. They found that the type and concentration of plasticizer had a considerable impact on the films made of the bioplastic's mechanical and thermal properties.
- **X. Wang et al. (2018)** compared the properties of bioplastics from human hair and feather keratin, finding that human hair bioplastics had higher transparency and mechanical properties and prepared bioplastics films from keratin extracted from both human hair and feathers, and compared the properties of the resulting bioplastics films. They found that the human hair bioplastics films exhibited higher transparency and better mechanical properties than the feather bioplastics films.

- **W. Zhang et al. (2019)** investigated that bioplastic films using keratin extracted from human hair and characterized the films using FTIR, XRD, SEM, and tensile strength testing. The study found that by including sorbitol as a plasticizer improved the mechanical qualities, as well as great transparency and strong tensile properties.
- **S. Gómez-Meléndez et al. (2019)** prepared bioplastic films using keratin extracted from human hair and characterized the films using FTIR, XRD, and SEM. The study found that the addition of glycerol as a plasticizer improved the flexibility of the films, and the films exhibited good mechanical properties.
- **Zhang et al. (2019)** investigated the use of human hair keratin as an energy source for the preparation of transparent bioplastics. Using a variety of reducing agents, the researchers isolated keratin from human hair, and the collected keratin was then dissolved in acetic acid to create a solution. Afterwards, a casting machine was used to cast the solution into films, which were subsequently tested for tensile strength, thermal stability, and water resistance. The findings demonstrated that the bioplastic sheets exhibited excellent mechanical qualities, high water resistance, and great transparency.
- **J. Wu et al. (2019)** prepared bioplastics from human hair keratin using a natural plasticizer, citric acid, resulting in bioplastics with high transparency and mechanical strength. The researchers used citric acid as a natural plasticizer to prepare bioplastics films from keratin extracted from human hair. The bioplastics films exhibited high transparency and mechanical strength, and the researchers found with the inclusion of citric acid improved the thermal stability of the bioplastics films.
- **S. Oikonomou et al. (2019)** produced bioplastics from human hair keratin and a biodegradable plasticizer, glycerol, resulting in transparent and flexible films. Synthesized bioplastics films from keratin extracted from human hair and a biodegradable plasticizer, glycerol. The resulting bioplastics films were transparent and flexible, and the researchers found that The inclusion of glycerol increased the films' mechanical characteristics.

- **H. S. El-Sabbagh et al. (2020)** used a mixture of glycerol and urea as plasticizers to prepare bioplastics from human hair keratin with high transparency and mechanical strength. Researchers investigated the use of a mixture of glycerol and urea as plasticizers in the preparation of bioplastics from keratin extracted from human hair. The study found that the resulting bioplastics had high transparency and mechanical strength, indicating the potential of the glycerol-urea mixture as a plasticizer for human hair bioplastics.
- **Gupta et al. (2020)** research included a project to create bioplastic films utilising keratin taken from human hair. The keratin was isolated from human hair by the researchers using a variety of reducing agents, and the retrieved keratin was then dissolved in formic acid to create a solution. Using a spin coating process, the solution was cast into films, and the films' thermal stability, mechanical characteristics, and water vapour permeability were all evaluated. The outcomes demonstrated that the bioplastic films had good mechanical and thermal durability as well as high transparency and low water vapour permeability.
- **Pramanik et al. (2021)** explored the use of human hair keratin in the synthesis of bioplastic films for food packaging applications. Using sodium sulfite and sodium hydroxide, the researchers isolated keratin from human hair, and the collected keratin was then dissolved in formic acid to create a solution. Using a casting machine, the solution was transformed into films, and the films' mechanical characteristics, water vapour permeability, and thermal stability were assessed. The findings demonstrated that the bioplastic sheets were acceptable for food packaging applications due to their superior mechanical capabilities, low water vapour permeability, and strong thermal stability.
- **A. Rahman et al. (2021)** prepared bioplastics from keratin extracted from human hair and chitosan, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and chitosan, a biodegradable and antimicrobial polymer. The researchers prepared bioplastics using a solution casting method

and tested the mechanical, thermal, and optical film's characteristics. According to the findings, the bioplastics exhibited superior mechanical qualities, including good tensile strength, modulus, and elongation at break, and they also had a high degree of transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and chitosan.

- **F. Zhang et al. (2021)** synthesized bioplastics from keratin extracted from human hair and modified starch, resulting in films with high transparency and biodegradability. To prepare bioplastics from keratin extracted from human hair and modified starch, a renewable and biodegradable material. The researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, and optical a film's characteristics. According to the findings the bioplastics had high transparency and biodegradability, with the degradation rate increasing with increasing starch content.
- **R. Natarajan et al. (2021)** synthesized bioplastics from keratin extracted from human hair and polyvinyl alcohol, resulting in films with high transparency and good mechanical properties. bioplastics from keratin extracted from human hair and polyvinyl alcohol, a biodegradable and water-soluble polymer. The scientists created bioplastics by solution casting them, and then they evaluated the films' mechanical, thermal, and optical characteristics. According to the findings, the bioplastics exhibited good mechanical properties, including good tensile strength, modulus, and elongation at break, and they also had a high degree of transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and polyvinyl alcohol.
- **Y. Liu et al. (2021)** prepared bioplastics from keratin extracted from human hair and citric acid, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and citric acid, a natural plasticizer and researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, and the films' optical qualities. The findings revealed that

the bioplastics exhibited excellent mechanical properties, including high transparency and strong tensile strength, modulus, and elongation at break. The researchers attributed the properties to the plasticizer's ability to enhance the intermolecular interactions between the keratin molecules.

CHAPTER-3

AIM & SCOPE

3.1 AIM

- The purpose of this project is to investigate if it is feasible to create functional protein Biofilms from human hair debris.
- Our study's goal is to investigate the keratin-based bioplastic films' characterisation, optimization, and prospective applications. We shall test the antibacterial activity of biofilm in this study.
- This research is based on the "Synthesis and characterisation of keratin based bioplastic film using human hair" riches from waste principle.

3.2 SCOPE

- In biological applications and in the food packaging industries
- Raw materials for various hygiene goods, such as nappy products' superabsorbent materials
- In agricultural sectors and tissue engineering (e.g., replacement of artificial hip joints, artificial skin and hearts).
- Used in the fashion sector as artificial skin replacement and breast implants.
- In the pharmaceutical sector (e.g., transdermal drug delivery systems, wound dressing, and medication delivery).
- It is utilised in composite materials such as reinforcement for building materials and superconducting system composites.

CHAPTER-4

MATERIALS AND METHODS

4.1 OVERALL PROCESS

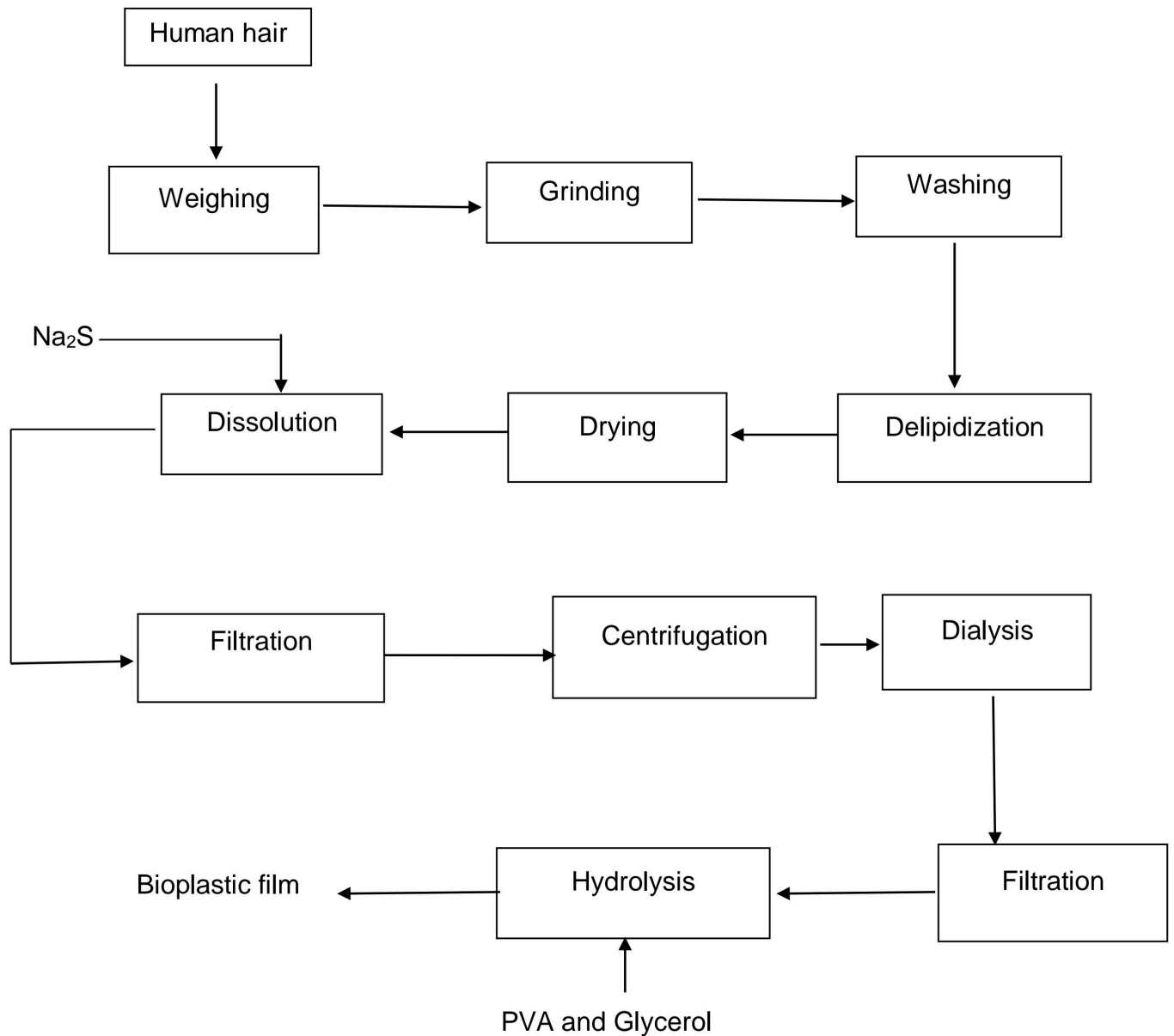


Fig.4.1; Overall process of keratin-based bioplastic film production

4.2 SELECTION OF MATERIALS

The unwanted human hairs were gathered from a nearby salon in OMR, Chennai, Tamil Nadu. Chemicals from Mercury Chemicals, Salem, included sodium sulfide, sodium hydroxide, ethanol, chloroform, glycerol, copper(II) sulphate, and benzoic acid, an antibacterial agent. The solution and washing were made with the distilled water.

Table 4.1: Usage of chemicals

S.No	Chemical used	Manufacturer
1	Ethanol	NICE chemicals
2	Chloroform	NICE chemicals
3	Glycerol	NICE chemicals
4	Sodium sulfide	NICE chemicals
5	Copper(II) sulphate	NICE chemicals
6	Benzoic acid	NICE chemicals
7	Polyvinyl alcohol	NICE chemicals
8	Sodium hydroxide	NICE chemicals
9	Distilled water	NICE chemicals

4.3 PRE-TREATMENT OF HUMAN HAIR

Before the keratin was taken out from the human hair, a few steps were taken. The following steps were part of the pre-treatment process.

- Weighing
- Grinding
- Washing
- Delipidization
- Drying

4.3.1 Weighing

First, the used human hairs were weighed. In the process of extraction, human hair with a total weight of about 50gm was used. The difference in weighing before and after washing was used to calculate the ratio of impurities to human hair.

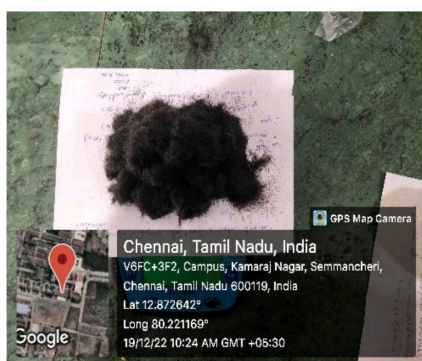


Fig.4.2;a)Human hair weighing



b)Human hair cutting

4.3.2 Grinding

Using a grinder, the provided human hairs were cut into tiny (0.2–0.3 cm) pieces. The unwanted materials, such as stones, papers, etc., were taken out before the grinding process.

4.3.3 Washing

Before using them to make plastic, the human hairs were thoroughly cleaned with soap and distilled water to get rid of any impurities like dirt and other unwanted materials. The human hairs were first cleaned, and then ethanol was applied to kill any remaining microorganisms. Proper washing was carried out to prevent contamination of the finished product. The cleaned hairs were then dried outside in the sun for 24 hours.

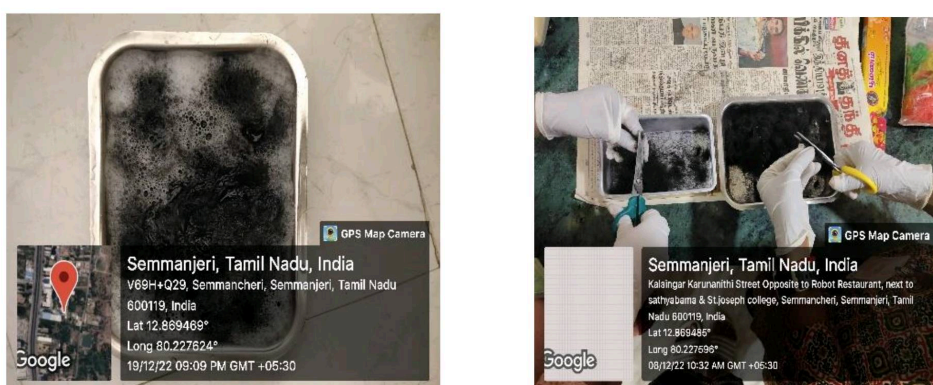


Fig.4.3; Human hair washing with soap

4.3.4 Delipidization and drying

The process of delipidizing involves removing lipids or lipid groups from a given substance. After being delipidized (1:2, v/v) with ethanol and chloroform for 24 hours at room temperature to remove the lipids from the hair, the hairs were then dried in a hot oven before the protein extraction step as in figure 4.4. To delipidize human hair in an industrial setting, lipid extractors and flash dryers will be used.



Fig.4.4; a) Ethanol and delipidization process b)Drying of treated hair

4.4 KERATIN EXTRACTION FROM HUMAN HAIR

There are several methods for extracting keratin using human hair, including the The sodium sulphide approach, the peracetic acid method, the thioglycolic acid method, and the urea method. We use the sodium sulphide method for extracting keratin because it is an easy and affordable process. This extraction process includes various processes such as dissolution, filtration, centrifugation, dialysis, and so on.

4.4.1 Dissolution of human hair with sodium sulfide

A total of 25 g of cleaned, dried, and delipidized hairs were added to one litre of a (100, 300, and 500 mM) sodium sulphide solution as shown in table 4.2 and heated at 50–60°C for 5–6 hours while being continuously stirred with a magnetic Stirrer. Nearly all human hairs have been dissolved after 6 hours, and the remaining hairs were separated by a filtration process.

Table 4.2:Concentration of sodium sulfide

Weight of human hair (g)	Concentration of sodium sulfide (mM)	Weight of sodium sulfide (g)
25	100	5.49
25	300	16.49
25	500	27.49

4.4.2 Filtration process of dissolved hair

The dissolved solution was filtered by running it through whatman filter paper. The retentate were eliminated following the filtration procedure.



Fig.4.5; a) Dissolution of hair



b)Filtration of human hair

4.4.3 Centrifugation of filtrate

Following filtration, the filtrate solution underwent a 20-minutes centrifugation at 5000 rpm. The particles contained in the supernatant were removed and discarded. Hydrochloric acid solution was used to bring the supernatant's pH to 3.5.

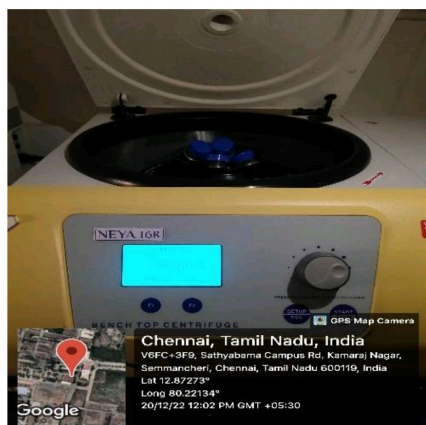


Fig.4.6;a) Centrifugation process

b) Supernatant liquid

4.4.4 Dialysis of supernatant liquid

For three days, the supernatant was dialyzed using cellulose tubular membrane against 1L of distilled water. The supernatant was first placed inside a cellulose tubular membrane, which was then tightly sealed on both ends as shown in figure 4.7. This cellulose tubular membrane was added to a 1L solution of distilled water, with the outer distilled water being changed three times daily. The excess sulphur in the precipitated solution was removed using this procedure.

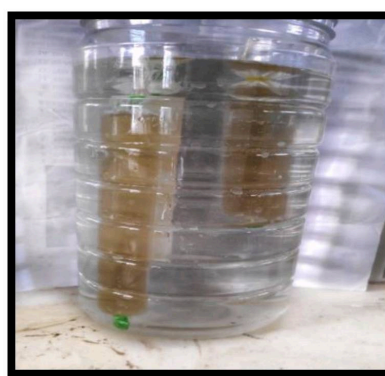


Fig.4.7; a) Liquid in Cellulose tubular membrane b) Dialysis process

4.4.5 Filtration and Freeze drying of keratin sample

The dialyzed sample was filtered using Whatman filter paper to obtain the protein fraction. Prior to use, this protein will be kept at 4°C. The given keratin protein was examined using FTIR as well as the biuret test to confirm its identity.



Fig.4.8; a) Filtration of dialyzed sample

b) Extracted keratin sample

4.5 CHARACTERIZATION OF KERATIN SAMPLE

Using FTIR in the attenuated reflectance mode, the functional group present in keratin protein was identified.

4.6 DETERMINATION OF EXTRACTION YIELD

The initial weight of the discarded human hair and the total dry mass of the keratin sample were used to calculate the obtaining yield. (Yin et al., 2013).

$$\text{Extraction yield (\%)} = W_1/W * 100$$

Where W= Initial mass of the human hair

W_1 = Mass of the freeze dried sample . The weight difference was computed.

4.7 DETERMINATION OF MOISTURE CONTENT OF KERATIN SAMPLE

Before being heated at 105 °C for 3 hours to a constant weight, a 2g sample was weighed precisely in a known weight crucible. The sample was then put in a dehydrator to cool, and the weight was then determined again(Prasetyo et al., 2017b).

$$\% \text{ moisture content} = (W_s - W_1) / W_s * 100$$

where

W_s - sample weight, (g)

W_1 - sample weight after drying. (g)

4.8 DETERMINATION OF COLOUR, TEXTURE AND WATER SOLUBILITY OF KERATIN

A physical eye examination was used to determine sample colour and texture. 2 g of the sample were boiled at 90°C in 25 mL of hot water to determine their solubility in hot water.

4.9 BIURET TEST

It is also referred to as Piotrowski's test and is a chemical procedure for determining whether peptide bonds are present or not. The presence of keratin protein can be verified using the biuret reaction(Shaik & Author, 2019).

4.9.1 Biuret reagent preparation

Dissolve 100 ml can be made by dissolving 1 g of copper sulphate in distilled water. Stir carefully to combine 100 ml of demineralised water with 10 g of sodium hydroxide pellets.

4.9.2 Protein sample preparation

For the protein solution add 1g of dried keratin powder sample and mix it with 100 ml distilled water.

4.9.3 Procedure for biuret test

Add 2ml of NaOH to 2ml of protein sample then add 3-4 drops of CuSO₄. A purple-violet colour is a positive result.

4.9.4 Observation of colour change

- No colour change → protein not present
- Colour change (turns into deep purple) → protein is present

4.10 PRODUCTION OF BIOPLASTIC FILM

4.10.1 Methodology 1

➤ Production of bioplastic using gelatin

About 300-450 ml of water were boiled to the boiling point, and then 10–12% of starch by weight were added to make the bioplastic film. The development of plastic film was achieved by blending the ingredients to achieve uniformity and prevent lumping. This process caused the molecules to line up and bind in an orderly manner to create a stronger substance. 9–12% by weight of plasticizers derived from glucose were added to the boiling solution to increase the blending and moulding properties. The highest tensile strength, tensile modulus, and thermal stability are anticipated from the plasticizers. The polysaccharides found in the keratin removed from human hair serve as the foundation for bioplastics. Previously processed 9–12 grammes of the keratin were added to the boiling mixture of starch and plasticizers, and then 10-12% by volume of glycerol was added to trap the water molecules in the starch chains, making the mixture less crystalline and less brittle. The addition of citric acid, which is 40 to 50 percent by volume of glycerol, increased the tensile strength even more. The required addition of 0.5 M NaOH helped to consistently maintain the pH of the bioplastic formulation. A 1:1 equal molar (0.5M) mixture of KOH and CH₃COOH was added to the bio-plastic to increase its resistance to both rodent and fungal attack following several trial formulations that demonstrated poor resistance to these attacks despite having the highest tensile strength available from the bioplastic. The right amount of walnut shell powder (3-5gms) and 30% by volume of 0.5M HCl were added to the bioplastic to strengthen it even more and improve uniform bonding.

5 to 8 ml of acetic acid source were added to increase solubility, and 20 to 23 percent by weight of gelatin was added to increase bioplasticity. All these components were added and mixed in regular manner in a boiling temperature of water as depicted in figure 4.9 to get a solution for the production of bioplastic.

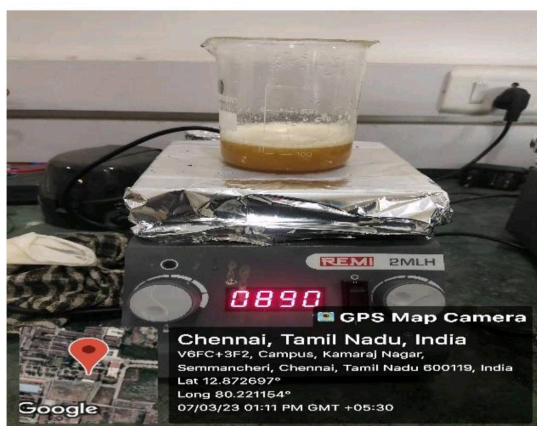


Figure 4.9 Solution of keratin mixture

➤ Drying and finishing process of bioplastic film

The mixture containing all the ingredients is mixed well and uniformly using a spatula. Continuous mixing is done to avoid any kind of lump formation and uniformity in mixture. Sieving of the mixture is done to remove all the foam and bubbles in the mixture. Then the mixture is transferred into a flat bottom tray. The tray is placed at a temperature of 20°C in a dark place to allow drying. The tray is left undisturbed for 60 hours. Completely dried bioplastic layer is slowly separated from the tray and used for various applications such as making of gaskets, insulation of wires, bioslippers etc..

➤ Trial runs

Table 4.3: No of Trial runs and materials used in Methodology 1

No of trials →			
Amount of materials used in trials ↓	Trial 1	Trial 2	Trial 3
Demineralized water(ml)	100	100	100
Starch soluble (g)	15	20	15
D-Sorbitol(g)	15	20	15
Keratin (g)	7	10	12
Glycerol (ml)	4.8	12	6
Citric acid(g)	4	3	2
Walnutshell powder(g)	3	3	3
0.5 M NaOH(ml)	2	5	2
0.5 M HCl(ml)	2	5	2
Gelatin (g)	10	10	7

4.10.2 Methodology 2

➤ Hydrolysis of keratin powder

To make the bio-plastic film, 15g 100 cc of water are dissolved with of PVA. and placed in a magnetic stirrer at 80°C for one hour. The 0.5g of keratin sample is mixed with 60 ml of 0.2 M NaOH Solution, followed by 10 ml of glycerol and 30 ml of PVA. The mixture is then continuously stirred in a magnetic stirrer set at 60 °C for two hour as shown in figure 4.10.

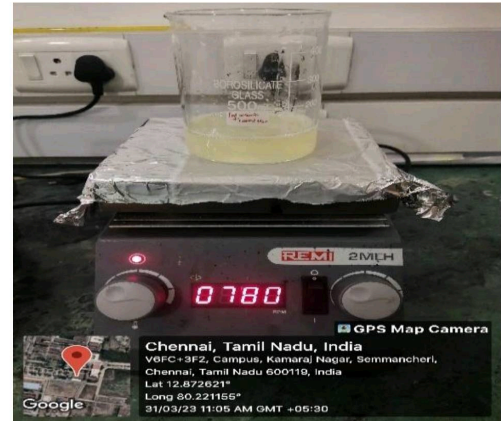
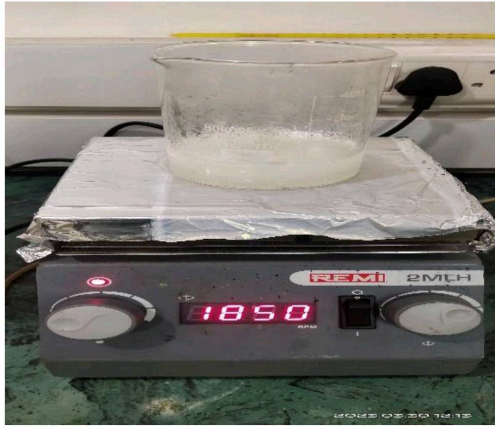


Fig.4.10; a) Polyvinyl alcohol solution

b)Hydrolysis of keratin solution

- Drying of bioplastic film

A 19*10 mm diameter petriplate was filled with the prepared solution, and the plate was then placed in a oven for 24 hours. The film were stripped out from the petriplate and stored for further examination.

- Trial runs

Table 4.4:No of Trial runs and the materials used in Methodology 2

No of trials →	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Materials used ↓									
PVA(g)	15	15	15	15	15	15	15	15	15
Demineralized water(ml)	100	100	100	100	100	100	100	100	100
PVA(ml)	30	30	30	30	30	30	30	30	30
Glycerol(ml)	10	6	15	9	15	10	10	10	10
Benzoic acid(g)	-	-	-	-	0.5	0.5	-	0.5	0.5
NaOH solution(ml)	60	60	60	60	60	60	60	60	60
Keratin powder(g)	0.03	0.04	0.05	0.5	0.1	0.5	0.3	0.5	0.5
Time taken(hr)	2	2	2	2	1:30	2	1:30	3	3
Remarks of product						Good	Good		

4.10.3 Methodology 3**➤ Preparation of bioplastic film**

The extracted Keratin weighed 1g should be taken separately in a beaker. Add 1 g of polyethylene glycol and 1 ml of glycerol. Glycerol is a plasticizer. 1 g of carboxy methyl cellulose should be added. This mixture is put into a beaker with 100 ml of water in it. The mixture is heated under 80°C at 100 rpm in a magnetic stirrer for a minimum of two hours. The obtained sample is then put in a dryer, (ideally one that also contains

magnesium nitrate hexahydrate), and the product is obtained at the desired consistency. The sample is sent for processing to injection moulding.

➤ Trial runs

Table 4.5 No. of trial runs and the materials used in Methodology 3

No of trials →	Trial 1	Trial 2
Materials used ↓		
PVA	1	1
Glycerol	5	10
Polyethylene glycol	1	1
Keratin sample	1	2
Carboxy methyl cellulose	1	1
Distilled water	100	100
Time taken	1:30	1

CHAPTER – 5

RESULTS AND DISCUSSION

5.1 DELIPIDIZATION OF HUMAN HAIR

After delipidization the surface oil and internal lipids from the hair was removed. The removal of oil ensures that the extracted keratin will be of high quality and purity. The weight before the delipidization process and after the process also varies and it makes sure that removal of oil takes place in this process.

5.2 DISSOLUTION OF HUMAN HAIR

In this process the sodium sulfide makes the human hair to dissolve completely. The yield of keratin is more in 500 mM concentration because the hair got completely dissolved in sodium sulfide when compared to other concentration. The different volume of sodium sulfide taken in this process gives the yield in increasing order which makes us to understand the importance of sodium sulfide usage.

5.3 DIALYSIS OF SUPERNATANT LIQUID

The sulphur got removed from the solution when it get dialyzed against distilled water as a buffer solution. The removal of sulphur in keratin extraction helps to prevent the reduction of mechanical properties, color changes etc.. By dialyzing, the small impurities and unwanted molecules also removed from the keratin and make it pure and suitable for industrial and biomedical application.

Table 5.1: Components of Keratin

Keratin components	Composition
Amino acids	20%
Peptides	15%
Minerals	5%
Disulfide bonds	10%

5.4 EXTRACTED KERATIN SAMPLE



Fig.5.1; Sample of keratin powder

5.5 YIELD % OF EXTRACTED KERATIN

The yield of keratin based dissolution of human hair in sodium sulfide solution. It shows maximum yield at highest concentration of 500 Mm.

Table 5.2: Calculated yield percentage

Serial no	Concentration of sodium sulfide used(mM)	Initial weight of human hair taken	Amount of extracted keratin powder(g)	Yield %
1	100	25	16.3	65.2
2	200	25	19.2	76.8
3	300	25	22.4	89.6

5.5.1 Effect of concentration and time on yield % graph

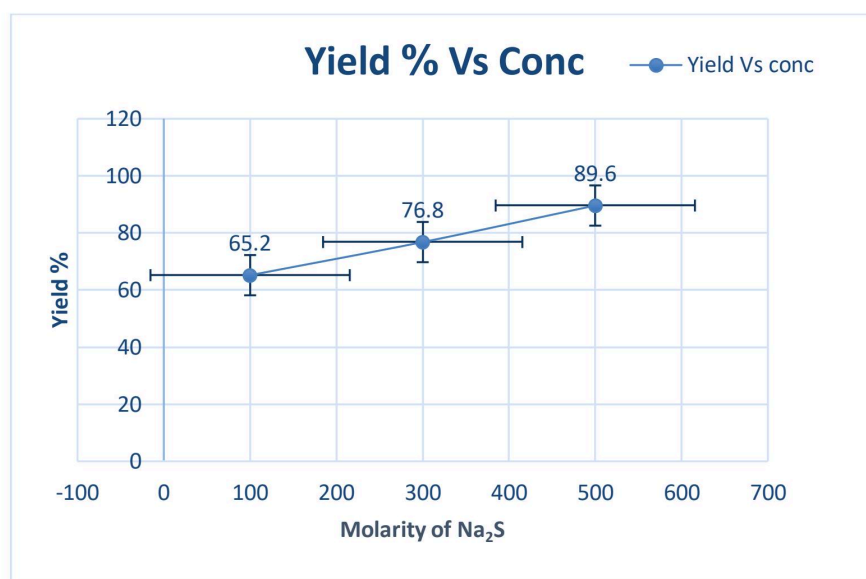


Fig.5.2; Yield % Vs Concentration graph

The human hair showed maximum solubility at 6 hr. After 6 hr there is no change in the given solution. In this figure, the graph showed the increasing order of keratin yield with respect to the increase in time and it remains constant after 6 hr.

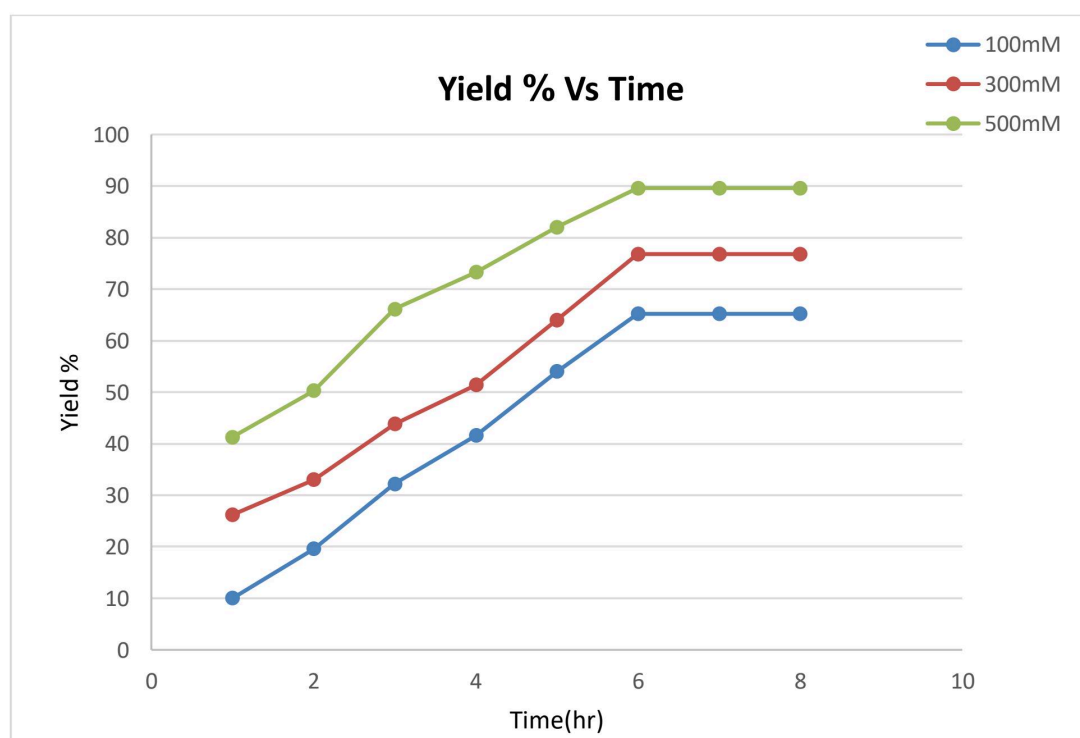


Fig.5.3; Yield % Vs Time graph

5.6 SOLUBILIZATION OF HUMAN HAIR

Chops of human hair were dissolved in Na_2S at 0.1, 0.3, and 0.5M concentrations. With 0.5M Na_2S , the human hair fractions were most soluble. The human hairs were partially dissolved at lower concentrations of 0.1 and 0.3M. The keratin from 25g of dry human hair yielded 65.2%, 76.8%, and 89.6% at 0.1, 0.3, and 0.5 M. The production was of keratin increased, with higher Na_2S concentrations. The human hair fractions' solubility peaked at 6 hours. The given solution has not changed after 6 hours.

5.7 KERATIN CONFIRMATION TEST

5.7.1 Confirmation of protein using Biuret test

The given solution changed to deep violet purple in the given figure 5.4. It demonstrates that keratin protein exist.

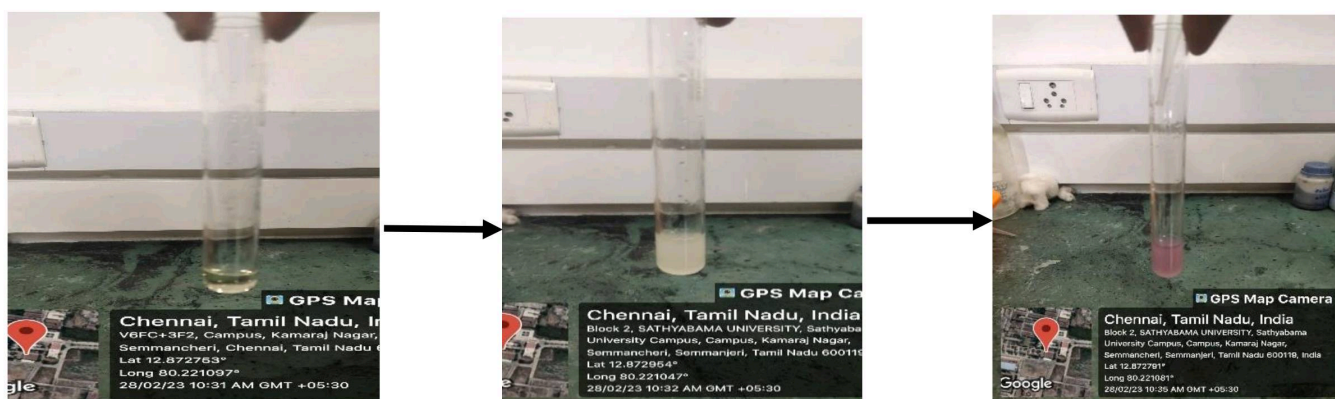


Fig.5.4; Changes in the sample after using biuret reagent

5.7.2 Characterization of keratin sample

It provided the useful details regarding molecular structure and structure of protein. The intensity and shape of these bands provide the information on the secondary structure and alterations in conformation in the keratin sample.

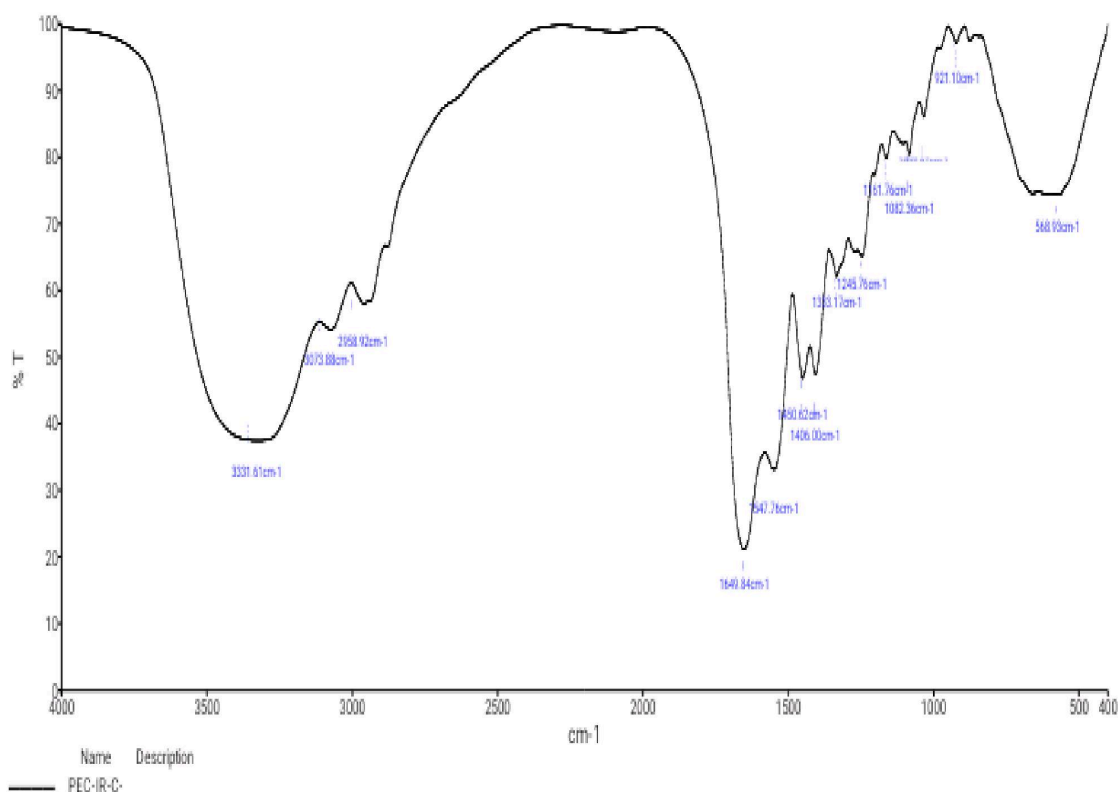


Fig.5.5; FTIR spectrum of produced keratin powder

Figure 5.5 displays the FT-IR spectrum of the keratin that was generated. The findings revealed the Amide A, 1, 2, and 3 groupings. Amide A is the name of a nitrogen hydrogen bond that demonstrated the existence of a group made up of amide and had a broad and weak mono absorbance zone at 3331.6 cm^{-1} . Amidates I through III investigated the protein structural configurations. The spectra of the samples revealed the absorption bands of C=O at 1649.84 cm^{-1} (amide 1), the nitrogen hydrogen bending at 1547.76 cm^{-1} (amide 2), and C-N bonds and C-O bending at 1245.76 cm^{-1} (amide 3).

Table 5.3:Major functional groups identified in keratin

Frequency ranges (cm^{-1})	Observed range (cm^{-1})	Functional groups
3400-3300	3331.6	N-H Stretching vibration Presence of amide A
3300-2500	3331.61	O-H Stretching vibration presence of

		carboxylic acid
3200-2700	3073.88	O-H stretching vibration presence of alcohol and phenol
3000-2840	2958.92	C-H Stretching vibration Presence of alkane
1630-1710	1649.84	C=O Stretching vibration Presence of amide-I
1550-1650	1547.76	N-H blending vibration Presence of amide-II (amine primary)
1415-1380	1406.00	S=O Stretching vibration Presence of sulfate
1350-1300	1333.17	S=O Stretching vibration Presence of sulfone
1250-1020	1245.76	C-N & C-O Stretching vibration Presence of amide-III
1205-1124	1161.76	C-O Stretching vibration Presence of tertiary alcohol

5.8 DETERMINATION OF MOISTURE CONTENT OF EXTRACTED KERATIN:

The moisture content of the keratin sample is calculated to be 13.5%.

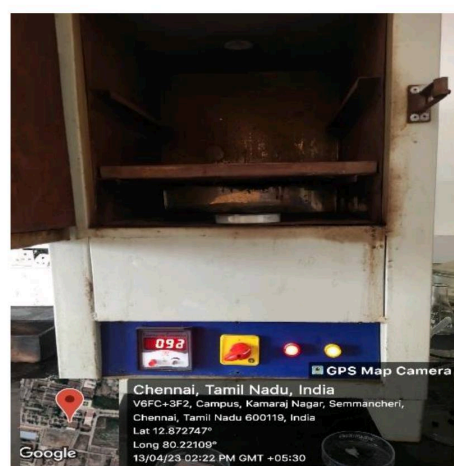
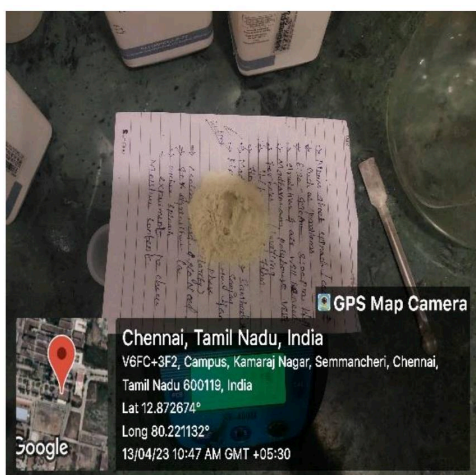




Fig.5.6; Analysis of moisture content

5.9 COLOUR,TEXTURE AND WATER SOLUBILITY

Using hand feel and physical eye examination , it was observed that the keratin was finely powdered material and yellowish brown in colour.keratin is completely insolble in cold and hot water as shown in figure 5.7.

Table 5.4:Colour, Texture and Solubility

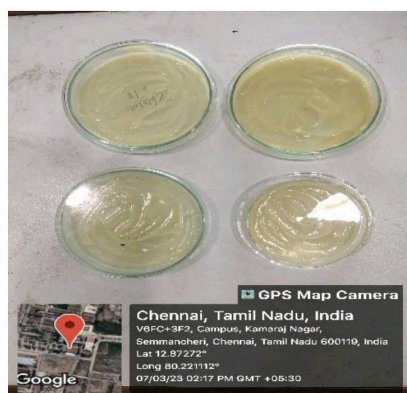
Texture	Fine powdered
Colour	Yellowish brown
Water solubility	Insoluble
Moisture content	13.5%



Fig.5.7; Solubility of keratin powder in water

5.10 BIOPLASTIC FILM TRIALS

5.10.1 Trial run pictures of Methodology 1



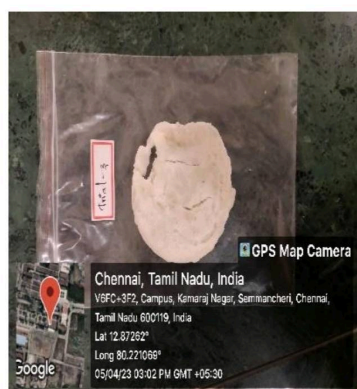
Trial 2



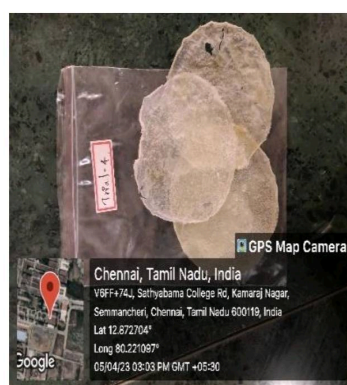
Trial 3

Fig.5.8; Products obtained from Methodology 1

5.10.2 Trial run pictures of Methodology 2



Trial -3



Trial- 4



Trial- 5



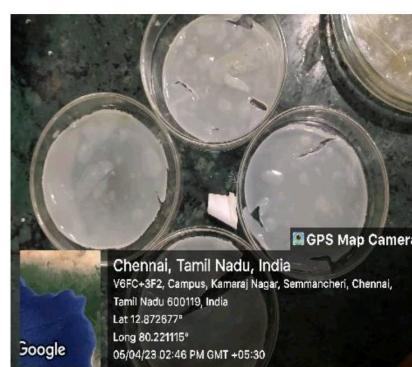
Trial - 6



Trial - 7



Trial - 8



Trial - 9

Fig.5.9; Products obtained from Methodology 2

5.10.3 Trial run pictures of Methodology 3



Trial 2

Fig.5.10; Products obtained from Methodology 3

5.11 FINAL OPTIMIZED METHOD FOR BIOPLASTIC FILM PRODUCTION

After trying three methods for the production of bioplastic film, the finally optimized method is the method 2 i.e hydrolysis of keratin powder. In this process keratin breaks down to soluble form and it helps in the production of bioplastic film. On the first 2 trial film is produced it got stick into the beaker. In trial 3, 4 and 5, the bioplastic film is produced but it got affected by fungal activity. To prevent the microbial attack, antibacterial agent i.e benzoic acid, is added. In trial 6, the film produced in this concentration was smooth and has a good colour, strength and mechanical properties like tensile strength, hardness etc., The first and second concentration keratin powder were used in the trial 8 and 9 and it does not come out well. It has less elasticity when compared to the third concentration keratin powder. The film produced in the method 3 was sticky and it has more moisture content even it is dried in the oven for 2 long days. This moisture leads to a fungal attack easily. The film produced in method 1 also not good when compared to method 1.

5.11.1 Optimized bioplastic film

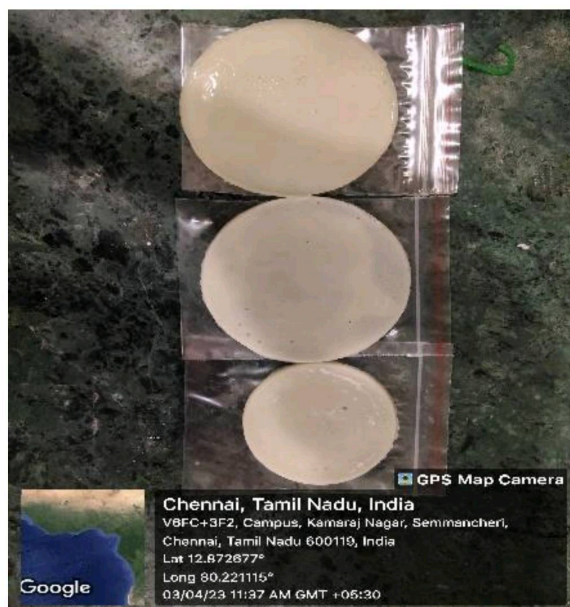


Figure 5.11 Bioplastic film

5.12 CHARACTERIZATION OF BIOPLASTIC FILM

5.12.1 Scanning electron microscope

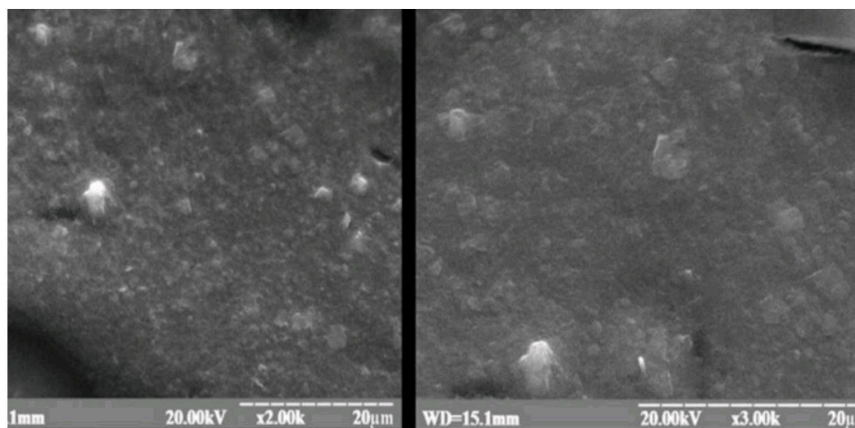


Fig.5.12; SEM Image of Optimized film

Figure 5.12 displays the findings of a SEM examination of keratin based bioplastic film. It has extremely good suitable morphologies that are free of cavities, edges, and holes. The occurrence of chemical interactions between keratin and glycerol was therefore attributed to the components' strong bonding. As a result, the glycerol contributed to the creation of a homogeneous mixture with visible plasticization in the keratin matrix. According to SEM analysis of the specimen's structure, the bioplastic's lightness at its smooth surface implies the presence of bonded structures. Their smooth surface morphology with fissures makes this clear, demonstrating their robust character

5.12.2 X-Ray Diffraction

Using an XRD system with copper K-ray radiation and generator parameters of 45 kV and 30 mA, the crystal structure of Keratin-based bioplastic films was assessed. The data for the dispersion angle differ from 5 to 50 were collected at a rate of 0.02/min. In accordance with Bragg's equation, the essential spacing was determined by the location of the peak in the XRD diagram:

$$n\lambda = 2d\sin\Theta$$

Where: n is the integer, d is the distance between the planes of crystals, θ is the dispersion angle, and λ is the electrons wavelength (Humphreys, 2013).

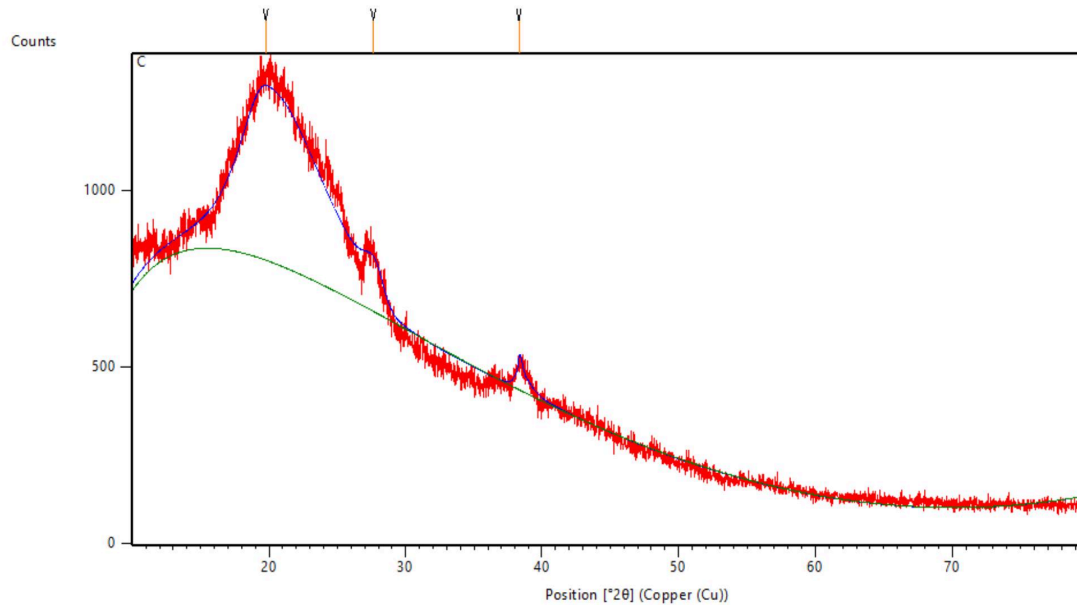


Figure 5.13 XRD graph of optimized graph

Figure 5.13 displays the XRD images of the keratin-based bioplastic film. It displayed primary peaks at $2\theta = 19.7$, 27.6 , and 38.3 showed the powerful crystalline characteristics. The following interlayer spacing decreased as the angle shifted upward, indicating an ordered structure for the blended component. The rise in d -spacing showed that the mixture's structure is less organised., which increases the difficulty of crystallisation. The term " d -spacing" refers to the distance between atoms. Bragg's equation states that as d -spacing is reduced, the value of 2 increases (Pope, 1997). The bioplastic film with the highest 2θ values and lowest d -spacing, as shown in Figure 4.13, also has the most ordered structure.

Table 5.5:X-ray diffractogram of synthesized Bioplastic film

Pos. [°2 θ]	Height [cts]	FWHM Left [°2 θ]	d-spacing [Å]	Rel. Int. [%]
19.7643	331.37	4.7345	4.48835	100.00
27.6219	112.22	3.0558	3.22680	33.87
38.3088	83.21	0.3591	2.34765	25.11

5.12.3 Fourier transform infrared spectroscopy

The figure 5.14 shows the FT-TR spectrum of keratin based bioplastic film. The findings revealed the Amide A, 1, 2, and 3 together. The occurrence of an amide unit was indicate by a broad and weak single absorption band for the N-H bond at 3437.36 cm^{-1} , which was named Amide A. Amidés I–III examined the protein

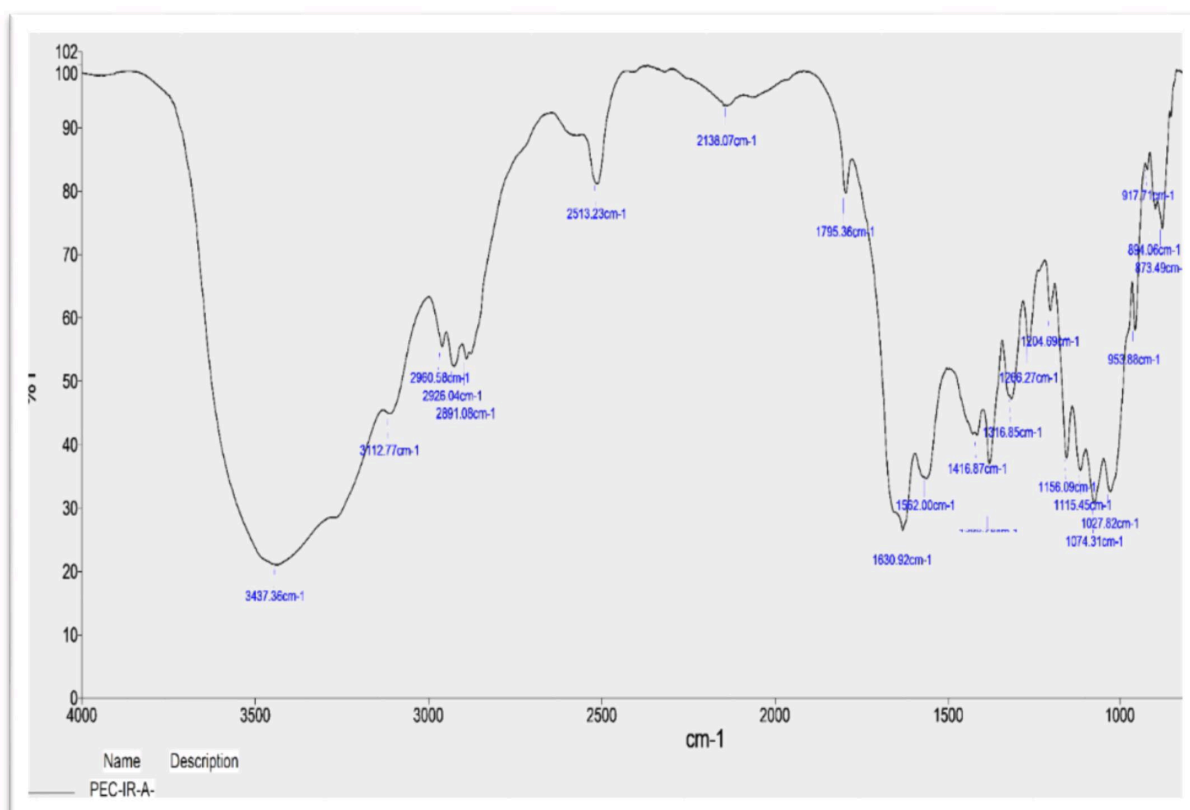


Fig.5.14;FTIR image of optimized film

conformational arrangements. The absorption bands of C=O at 1630.92 cm^{-1} (amide I), N-H bending at 1562.00 cm^{-1} (Amide II), and C-N and C-O stretching at 1204.69, 1027.82 and 1074.82 cm^{-1} (amide III) were showed in the wavelength of the sample. From the table 5.6, it shows the presence of functional groups like amine, amides, sulfone, alcohols etc. All of these groups indicates the presence of keratin and glycerol from the given bioplastic film

Table 5.6: Major functional groups identified in bioplastic film

Frequency ranges(cm^{-1})	Observed ranges(cm^{-1})	Functional groups
3400-3500	3437.36	Primary amine (amide A) present in the N-H stretching vibration
3300-2500	3112.77	O-H Stretching vibration presence of carboxylic acid
3200-2700	3112.77	O-H stretching vibration presence of alcohol and phenol
3000-2840	2960.58, 2891.08	C-H Stretching vibration Presence of alkane
1630-1710	1630.92	C=O Stretching vibration Presence of amide-I
1550-1650	1562.00	N-H blending vibration Presence of amide-II (amine primary)
1420-1330	1416.87	O-H blending vibration Presence of alcohol
1350-1300	1316.85	S=O Stretching vibration Presence of sulfone
1250-1020	1204.69, 1027.82, 1074.82	C-N & C-O Stretching vibration Presence of amide-III
1205-1124	1156.09, 1115.09	C-O Stretching vibration

		Presence of tertiary alcohol
900-990	953.88, 917.71	C=O blending vibration Presence of alkene

5.13 BIODEGRADABILITY TEST

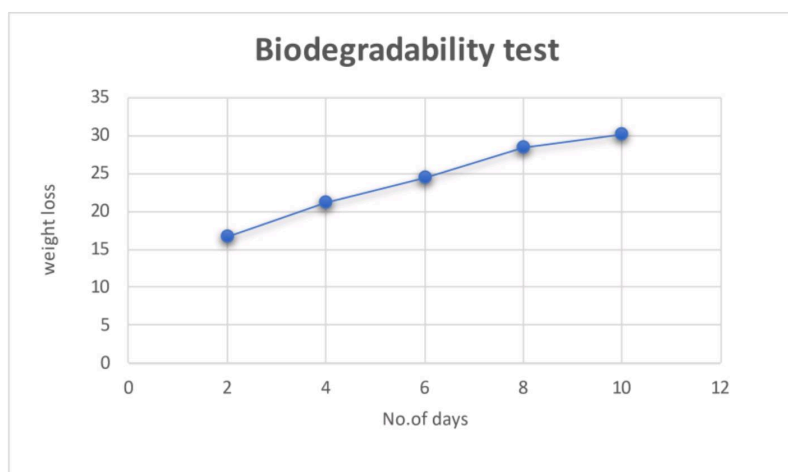


Fig.5.15; Biodegradability graph

Figures 5.15 and 5.16 display the outcomes of the biodegradability test conducted using the Soil Burial Test method. As can be seen, the reduction in weight of the bioplastic increases with the length of the burial period, which indicates the greater the degree of degradation. Every day, more weight was lost, reaching a peak of 30.2% on day 10. In linear polymers, chemical degradation reactions lead to a reduction in molecular mass or length chain minimizing. The size of this reduction in mass was caused by the composition of bioplastic, which was made of an organic substance that microbes could quickly digest (Harsojuwono et al., 2019).

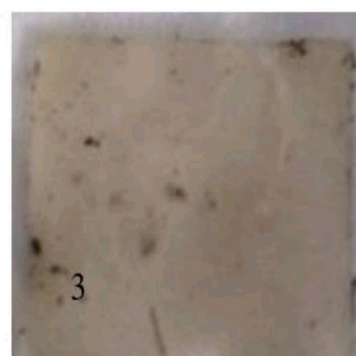




Fig.5.16; Soil burial Test. (1) Bioplastic on the day zero (2) size of bioplastic film was expanded on day two (3) More water and soil are entered into the bioplastic film on day four (4) Wide crack identified on bioplastic on day six (5) Some parts of bioplastic were diminished on day eight (6) most of the parts of bioplastics were completely degraded on day ten.

As depicted in Figure 5.16, the bioplastic sample started to physically change on day 4 where cracks were seen on the upper layer of the sample. The sample's form then started to break down on the eighth and tenth days. In contrast to another study [14], the sample's It shrank in size and became brittle and hard, after being buried for 90 days. Changes in macrostructure were significantly influenced by soil degradation. It's possible that the soil had more nutrients and that several microbes (fungi and bacteria) worked together in concert to damage the macrostructure of the bioplastic samples.

Table 5.7:Calculation of weight loss % of bioplastic film

Number of days	Weight loss %
Day 2	16.67
Day 4	21.1
Day 6	24.5
Day 8	28.4
Day 10	30.2

5.14 ANTIMICROBIAL ACTIVITY OF THE BIOFILM

In this study, the provided bioplastic film had antibacterial agents like benzoic acid added to it. An analysis of the biofilms on total bacterial count was done by the Tamilnadu Agricultural University. Bioplastic films without antibacterial agents (film₁) and bioplastic films with antibacterial agents (film₂) are the two types of biofilms that are analysed. According to the results, the total number of bacteria on films 1 and 2 is 7.2102 cfu/g and 2.36102 cfu/g, respectively. This finding demonstrates how antibacterial substances like benzoic acid can help to lessen microbial attack on biofilms.

Table 5.8: Total Bacterial count of bioplastic film

Types of bioplastic	Number of bacterial count
Film1	7.27×10 ² cfu/g
Film2	2.36×10 ² cfu/g

5.15 PROPERTIES OF BIOPLASTICS

5.15.1 Thickness of film

There were three different biofilm samples that were examined. With a Vernier calliper and fifteen unique measurement value on the film, the average film thickness was determined to the nearest 0.0001 mm. The outcome showed that the thickness of the films, which were recorded at 0.018, 0.019, and 0.024 mm, did not significantly differ from one another. Different amounts of keratin solution could be the cause of the variations in film thickness.

5.15.2 Density of film

There are three different pieces of biofilms was analyzed. The density of biofilm having recorded 1.39g/cm³, 1.43g/cm³, 1.51g/cm³. The density of film depends up on the thickness of film and area of biofilm. These outcomes imply that keratin would increase the biofilm's flexible and breaking strength. The molecular weight and density of the keratin, PVA, and plasticizers included in the biofilm may be responsible for the change in density.

5.15.3 Moisture content of film

Bioplastic with a higher concentration of keratin showed the highest moisture content of 22.78 ± 0.69 . As a result, bioplastic film with a higher moisture content can be used to pack food. These outcomes concur with those mentioned by (Prasetyo et al., 2017a)

5.15.4 Solubility of film

With a greater ratio of keratin content and amount of plasticizer compared to other bioplastics, the third demonstrated a poorer solubility in water. Normally, the presence of PVA would make the films more water-soluble, but in this case, the PVA was constant while the amounts of keratin and glycerol varied. The third concentration product with the greatest amount of keratin showed lowest solubility.

Table 5.9: Percentage of water solubility

Sample	Initial sample (g)	Final weight (g)	Solubility in water (%)
S ₁	0.302	0.264	12.52
S ₂	0.291	0.251	11.61
S ₃	0.312	0.276	11.53

5.15.5 Tensile strength of biofilm

The film's strength in tensile test shows the highest stress created during tensile testing (Sachdeva et al., 2017). The bioplastic made from first two concentration keratin sample and the amount of plasticizer used showed the minimum tensile strength of 7.67 ± 0.18 Mpa and 9 ± 0.20 . The maximum tensile strength of 10.4 ± 0.22 MPa was obtained on the third concentration film.

5.15.6 Young's modulus of elasticity

By measuring the Young's modulus, an item's or film's stiffness can be determined; the higher the value, the stiffer the film (Gonçalves et al., 2019). The maximum Young's modulus of 169 ± 0.88 MPa was obtained of the bioplastic film made from third concentration bioplastic film which exceeded the films created using the other two concentrations.

CHAPTER 6

SUMMARY AND CONCLUSION

Despite being merely composed of proteins, keratin is among the toughest materials found in nature and serves as an effective protective covering. It presents a huge opportunity for novel bioinspired designs with a deep comprehension of natural science, structure, and mechanism. The present chemical methods for getting keratin are efficient yet costly, making them less applicable for commercial uses. Therefore, there is a chance to create brand-new strategies for the efficient segregation of keratin. This experiment successfully created bioplastic films using human hair. The physicochemical and mechanical qualities of the films are significantly enhanced by the application of a moulding agent. The biodegradable film had the highest density, moisture content, transparency, and solubility in water of all the films. Their bending strength, and Young's modulus of elasticity are all increased by the presence of keratin. The presence of amides content was verified by FTIR, which also validated the chemical linkage and connection between the plasticizer and the matrix of polymers. The relative crystallinity of the bioplastic film is the greatest at 33.87%. The biological decomposition analysis shows that the bioplastic sheets degrade in a variety of situations, including composting, natural environments, and lab settings. A weight loss of 32.2% was achieved in lab settings after 10 days. As a result, the qualities that the films showed after the proper amount of plasticizer was added suggest that sustainable bioplastic production is possible. The film is ideal for packaging food due to its remarkable qualities, including low transparency, humidity, and solubility in water. Due to its excellent tensile strength and elastic modulus, it can also be utilised to produce rigid plastic containers and toys. The creation of valuable things can now employ other sources thanks to this research. In conclusion, keratin has been effectively extracted from human hair to create an artificial, organic-based bioplastic film. Even though the experiment showed remarkable bioplastic capabilities, the expected development was seen when keratin was included. Furthermore, the high crystal structure, good surface form, and thermal properties were validated by SEM and XRD analysis. Finally, there are numerous industrial uses for the developed keratin-based bioplastic film.

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SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED BIOPLASTIC FILM USING HUMAN HAIR

⁹ Submitted in partial fulfilment of the requirement for the award of
Bachelor of Technology Degree in Chemical Engineering

by

S. GAYATHRI (39190004)

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BONAFIDE CERTIFICATE

This is to certify that this project report is the bonafide work of S. GAYATHRI (39190004), N. MANASA (39190021) AND S.E. SUBASHINI (39190032) who carried out the project entitled **"SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED BIOPLASTIC FILM USING HUMAN HAIR"** under my supervision from November 2022 to April 2023

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DECLARATION

We S. GAYATHRI (39190004), N. MANASA (39190021) AND S.E. SUBASHINI (39190032) hereby declare that the Project Report entitled **“SYNTHESIS AND CHARACTERIZATION OF KERATIN BASED BIOPLASTIC FILM USING HUMAN HAIR”** done by me under the guidance of Dr.D. Prabhu M.S(By Research),Ph.D (Internal) and Dr.N.Nirmala M.Sc.,Ph.D (External) SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY, CHENNAI at is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in chemical engineering.

DATE:

PLACE: CHENNAI

SIGNATURE OF THE CANDIDATES

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ABSTRACT

Plastics are typically extremely strong polymers and one of the active ingredients that seriously harm the environment. The porosity of the soil is blocked by improper plastic bag disposal, which is problematic for groundwater resources. Plastics are not biodegradable, so leaving them in the environment as trash harms the environment permanently. The only way to address the growing threat of plastic waste to ecological sustainability is by creating biodegradable plastic, even from waste. Due to its stability against enzymatic degradation, keratin has gained interest and allowed for more predictable degradation profiles for tissue regeneration. Due to the presence of Keratin in human hair and it is highly microcrystalline, extremely strong, and resistant to both thermal as well as mechanical stress. The current study aims to extract keratin (protein) from human hair and use it to create a bioplastic film. Keratin is extracted using a sodium sulphide method, and bioplastic film is created by combining the extracted keratin solution with PVA and glycerol as a plasticizer. The keratin is confirmed by biuret test and FTIR. SEM, XRD, and FTIR will be used to characterise the prepared bioplastic film, and this paper will also discuss the material's mechanical, physical, and thermal properties.

Keywords: *Conventional plastic , Human hair , keratin , bioplastic film*

LIST OF CONTENT

CHAPTER NO	TITLE	PAGE NO
	ABSTRACT	v
	LIST OF CONTENT	vi
	LIST OF FIGURE	x
	LIST OF TABLE	xii
	LIST OF ABBREVIATION	xiii
1	INTRODUCTION	1
	1.1 PLASTICS	1
	1.1.1 An overview of plastic	1
	1.1.2 Plastic significance in modern culture	2
	1.1.3 Plastic production	5
	1.1.4 Impacts of conventional plastics	6
	1.2 BIOPLASTICS	8
	1.2.1 An overview of bioplastics	8
	1.2.2 Bioplastics as a closed loop approach	11
	1.2.3 Current state of bioplastics in India	12
	1.2.4 Biodegradation of bioplastics	13
	1.2.5 Sources of biomass	16
	1.3 KERATIN	18
	1.3.1 A Summary of keratin	18
	1.3.2 Human hair as a source of keratin extraction	20
2	LITERATURE SURVEY	22
3	AIM AND SCOPE	30
	3.1 AIM	30
	3.2 SCOPE	30
4	MATERIALS AND METHODS	31
	4.1 OVERALL PROCESS	31

	4.2 SELECTION OF PROCESS	32
	4.3 PRETREATMENT OF HUMAN HAIR	32
	4.3.1 Weighing of human hair	33
	4.3.2 Grinding of hair	33
	4.3.3 Washing of hair	34
	4.3.4 Delipidization and drying of human hair	34
	4.4 EXTRACTION OF KERATIN FROM HUMAN HAIR	35
	4.4.1 Dissolution of Human hair with sodium sulfide	35
	4.4.2 Filtration process of dissolved hair	36
	4.4.3 Centrifugation of filtrate	36
	4.4.4 Dialysis of supernatant liquid	37
	4.4.5 filtration and freeze drying of keratin sample	38
	4.5 CHARACTERIZATION OF KERATIN SAMPLE	38
	4.6 DETERMINATION OF EXTRACTION YIELD	38
	4.7 DETERMINATION OF MOISTURE CONTENT OF KERATIN SAMPLE	38
	4.8 DETERMINATION OF COLOUR, TEXTURE AND WATER SOLUBILITY OF KERATIN	39
	4.9 BIURET TEST	39
	4.9.1 Biuret reagent preparation	39
	4.9.2 Protein sample preparation	39
	4.9.3 Procedure for biuret test	40
	4.9.4 Observation of colour change	40
	4.10 PRODUCTION OF BIOPLASTIC FILM	40
	4.10.1 Methodology 1	40
	4.10.2 Methodology 2	42
	4.10.3 Methodology 3	44
5	RESULTS AND DISCUSSION	46
	5.1 DELIPIDIZATION OF HUMAN HAIR	46

5.2 DISSOLUTION OF HUMAN HAIR	46
5.3 DIALYSIS OF SUPERNATANT LIQUID	46
5.4 EXTRACTED KERATIN SAMPLE	47
5.5 YIELD% OF EXTRACTED KERATIN SAMPLE	47
5.5.1 Effect Of Concentration And Time On Yield% Graph	48
5.6 SOLUBILIZATION OF HUMAN HAIR	49
5.7 KERATIN CONFIRMATION TEST	49
5.7.1 Confirmation of protein using Biuret test	49
5.7.2 Characterization of keratin sample	50
5.8 DETERMINATION OF MOISTURE CONTENT OF EXTRACTED KERATIN	51
5.9 COLOUR,TEXTURE AND WATER SOLUBILITY	52
5.10 BIOPLASTIC FILM TRIALS	53
5.10.1 Trial Run Pictures Of Methodology 1	53
5.10.2 Trial Run Pictures Of Methodology 2	53
5.10.3 Trial Run Pictures Of Methodology 3	54
5.11 FINAL OPTIMIZED METHOD FOR BIOPLASTIC PRODUCTION	55
5.11.1 Optimized bioplastic film	55
5.12 CHARACTERIZATION OF BIOPLASTIC FILM	56
5.12.1 Scanning Electron Microscope	56
5.12.2 X-Ray Diffraction	57
5.12.3 Fourier Transform Microscope	58
5.13 BIODEGRADABILITY TEST	60
5.14 ANTIMICROBIAL ACTIVITY OF BIOFILM	62
5.15 PROPERTIES OF BIOPLASTIC FILM	62
5.15.1 Thickness of film	62
5.15.2 Density of film	62
5.15.3 Moisture content of film	63

	5.15.4 Solubility of film	63
	5.15.5 Tensile strength of film	63
	5.15.6 Young's modulus of elasticity	63
6	SUMMARY AND CONCLUSION	64
	REFERENCES	65

LIST OF FIGURES

Figure no	Name	Page no
1.1	Flowchart for plastic production	5
1.2	Classification of bioplastics	10
1.3	Lifecycle of bioplastic	11
4.1	Overall process of keratin – based bioplastic film production	31
4.2	a) Experimental setup of weighing human hair	33
	b) Experimental setup of cutting human hair	33
4.3	Human hair washing with soap	34
4.4	a) Experimental setup of delipidization process	35
	b) Drying of treated hair	35
4.5	a) Dissolution of human hair in sodium sulfide	36
	b) Filtration of human hair	36
4.6	a) Centrifugation process	37
	b) Supernatant liquid	37
4.7	a) Liquid in cellulose tubular membrane	37
	b) Dialysis process	37
4.8	a) Filtration of Dialyzed sample	38
	b) Extracted keratin sample	38
4.9	Solution of keratin mixture	41
4.10	a) Polyvinyl alcohol Solution	43
	b) Hydrolysis of keratin solution	43
5.1	Sample of keratin powder	47
5.2	Graphical representation of effect of yield on concentration	48
5.3	Graphical representation of effect of yield on time	48
5.4	Changes in the sample after using biuret reagent	49

5.5	FTIR spectrum of produced keratin powder	50
5.6	Analysis of Moisture content	51
5.7	Solubility of keratin powder in water	52
5.8	Products obtained from methodology 1	53
5.9	Products obtained from methodology 2	54
5.10	Products obtained from methodology 3	54
5.11	Final bioplastic film	55
5.12	SEM analysis image of optimized film	56
5.13	XRD analysis image of optimized film	57
5.14	FTIR analysis image of optimized film	58
5.15	Biodegradability graph	60
5.16	Soil burial test	61

LIST OF TABLES

Table no	Name	Page no
4.1	Usage of chemicals	32
4.2	Concentration of sodium sulfide	36
4.3	No of trial runs and materials used in methodology 1	42
4.4	No of trial runs and materials used in methodology 2	44
4.5	No of trial runs and materials used in methodology 3	45
5.1	Components of keratin	46
5.2	Calculated yield%	47
5.3	Major functional groups identified in keratin	51
5.4	Colour,texture and water solubility	52
5.5	X-Ray diffractogram of extracted bioplastic film	58
5.6	Major functional group identified in bioplastic film	59
5.7	Calculation of weight loss% of bioplastic film	61
5.8	Total bacterial count of bioplastic film	62
5.9	Percentage of water solubility	63

LIST OF ABBREVIATIONS

BPA	- Bisphenol
EPA	- Environmental Protection Agency
PBT	- Persistent,Bioaccumulative,Toxic
UV	- Ultra Violet
RGF	- Regenerative Cellulose Film
PVA	- Polyvinyl Alcohol
RPM	- Rotation Per Minute
SBT	- Soil Burial Test
SEM	- Scanning Electron Microscope
FTIR	- Fourier Transform Infrared Spectroscopy
XRD	- X-Ray Diffraction
ASTM	- American Society For Testing Material
PCL	- Polycaprolactum
PLA	- Polylactic Acid

CHAPTER-1

INTRODUCTION

1.1 PLASTICS

1.1.1 An Overview of Plastic

Every year, roughly 330 million pounds of plastic are manufactured worldwide. Nearly 280 million metric tons of plastic were produced globally in 2012 only, according to estimates. Over 130 million metric tons of plastics throughout that total have been reused or dumped in landfills. Plastic will find a habitat in people's everyday life among 150 million metric tons that are left. Meanwhile, the remainder of the plastics percentage ends up as trash in water bodies or lying on the surface of the earth. As a result, the plastic trash poses a serious environmental threat to modern society since it contains several hazardous substances and, if improperly managed or cleaned, pollutes the Earth's soil, the atmosphere, and water bodies. Plastics are mostly used in the food packaging (40%), engineering (20%), and automobile (8%) sectors, as well as in the production of home products.

The overwhelming majority of industrialized plastics have no capacity for biodegradable and, as a result, cause environmental issues owing to a rise in accumulation of solid waste. Plastics are utilised in a broad variety of items that have swapped and displaced several other substances, like paper, timber, stone, leather goods, glass, metals, and ceramics, according to their easy nature of manufacturing, affordability, durability, and resistant to chemicals, weather, and lighting. Plastics are a prevalent material in contemporary society and are used in everything from spacecraft to stationary objects. Plastic is generally tough, expandable, waterproof, lightweight material, and extremely affordable to create. (Obebe SB & Adamu AA, 2020)

These facts are the appealing characteristics that encourage the excessive consumption of products made of polymer. As a replacement, plastic that is entirely formed of hydrocarbon products is particularly useful and degrades slowly. Plastic-

Pollution Association claims that the attractiveness of the plastic products we use of a daily basis has caused inescapable behavioural issues to emerge, which have resulted in overconsumption. At a given time, this behaviour seriously pollutes the surroundings. Prior research on the use of plastic debris in the production of construction has been documented.

Bags made of plastic, that are frequently used for packaging, carrying veggies and meat, among other things, have a major adverse effects on the surroundings. Because material bags can stay in the atmosphere over a maximum of one thousand years without decomposing, the number of plastic packs that are produced each year rises. Large amounts of plastic bags that are discarded or partially accumulated pollute the environment, water, and fresh air, damaging the biosphere and adversely impacting the organisms that live there.(Bandini et al., 2022)

Plastics cause a tremendous environmental impact. Those are resilient, lightweight, as well as adaptable, yet they degrade slowly and can remain in the surroundings for thousands of years. Above are some of the various ways that plastics harm the environment(Bandini et al., 2022):

1. Pollution: Plastic debris is a major pollutant in garbage dumps seas, and other sources of water. Plastics can require thousands of years to disintegrate, generating hazardous compounds and microplastics that damage biodiversity and ecosystems.
2. Habitat destruction: Plastic pollution may devastate animal habitats especially reefs and habitats for marine turtles.
3. Climate change: Plastic manufacture and disposal result in the release of greenhouse gases, which exacerbate global warming.
4. Impacts on human health: Plastics can include toxic chemicals like BPA (bisphenol A), and phthalates, which may enter water and food supplies as well as the well-being of humans.

1.1.2 Plastic's significance in modern culture

Polymers are more complicated than metals, it may categorise as natural or artificial 'macromolecules' comprised of lower repeating components. Organic polymers are synthesized in wildlife, plants, and both microscopic and macro-organisms. Polyunsaturated proteins, lipids, nucleic acid sequences, & rubber from plants are among examples. Cellulose, a highly prevalent organic compound on the planet, is a readily identified natural polymer. Artificial materials are polymers that are created by humans. The most significant among synthetic polymers include polyolefins. Plastics made from synthetic materials are prevalent in the environment, finding various uses in many industries due to their helpful features (mechanical and physiological properties) and low cost, contributing to the improvement of the ease and standard living in our current industrial civilization.

Polymers are not biodegradable they can be used in a variety of applications. Perhaps the most astounding characteristic of polymers produced from natural goods is the fact that such ecologically unstable substances should serve as the foundation for environmentally lasting goods used in industry. Rubber tyres represent the fewest ecologically stable of every polymer before manufacture, although vehicle tyres exist in the external environment over a while after usage. The following are the main applications of referred to as non-degradable thermoplastics(Thompson et al., 2009):

1. Surface coatings and paintings:Natural 'drying lubricants' made from unsaturated has been utilized to generations to preserve heavy metals through rusting and timber from bacterial activity. The most significant 'environmentally friendly' innovations that have developed were connected coverings and printing that used oligomeric acrylate polymers.
2. At workplace and Home:Plastics have emerged as the latest material option for devices performing at room temperature, including food mixers, Hoover cleaners, hair dryers, tv consoles, desktops, word editors and various office equipment.
3. Transportation:Polymers are lighter than metals and ceramics. The current plastic dairy container weighs only a quarter of the capacity of a comparable glass bottle, which has a substantial impact on transportation costs. This

is widely held that milk delivered in recyclable glass cans is more environmentally friendly than single-trip containers made of plastic. Synthetic plastics are suited for this purpose due to their non-biodegradability. Polymers are also progressively switching traditional substances in machinery such as automobiles, aircraft, and watercraft, because of their long lifespans. All plastics having densities around one, but even the lightweight of regularly used metals, aluminium, is far heavier. It is projected that during the past ten years, 200-300 kilogram of conventional supplies in the normal vehicle would have been substituted by 100 kilograms of plastics, resulting a roughly 750 litre reduction in gasoline consumption throughout the usable lifespan of the vehicle. Furthermore, because the manufacturing of metals as well as glass requires more power than the generation of ordinary plastics, more polymers that could be utilised to replace alloys and glasses in automobiles, the less gasoline will be consumed in transportation. (Siracusa et al., 2008)

4. Agriculture: Plastics have affected rural areas due to their widespread used in farming, garbage disposal, and irrigation. Photo-degradable plastics might be ineffective because longevity of utilization is a significant consideration in these applications.
5. Packaging: Synthetic polymers have primarily been used as substitutes for standard materials, especially for packaging. Plastics are lightweight materials with excellent barrier characteristics against aquatic as well as water-borne microbes. They outperform glass in terms of hardness and robustness, resulting in less product loss while transportation. They safeguard the ecosystem not just from volatile goods, also from caustic or hazardous chemicals. Plastics made from petroleum-based products use significantly less labour and power than conventional substances (Thomas et al., 2012).
6. Public utilities: In the past few years, plastics have gained a more significant position in subsurface applications. In locations in which concrete or stainless steel were once employed, these include pipelines, ducting, and underneath chambers. Impermeable layers are now being used for a variety of new applications, such as the contamination of groundwater in storage tanks and

discharges in sanitary landfills, arrays and nets for soil stability, and underground electrical lines. Utilizing their capability to withstand biodegradation, synthetic materials are used in subsoil applications. An ever-growing component of utility delivery to commercial and home consumers is the underlying transportation of gas, electricity, and oil via pipeline(Meereboer et al., 2020).

7. Medicine and Biology:Polymers' biological stiffness and lightweight make them particularly appealing for future biomedical applications. Clinical applications are usual instances. Plastic prosthesis for replacement requires cutting-edge technology. However, in such application, the toughness with biocompatibility of every polymer under the severe circumstances to which products are exposed in use act as fundamental design criteria.

1.1.3 Plastic production

Oil and natural gas are just two of the numerous raw materials that are used in the production of plastics. Crude oil is first extracted, and after that it is processed to produce a variety of chemical compounds, such as ethylene, propylene, and benzene. Then, these chemical substances are utilised as a raw material for the manufacture of plastic. Figure 1.1 depicts the production of plastics in various ways.The three most popular processes for producing plastic are injection molding, blow molding, and extrusion(Kalita et al., 2021).

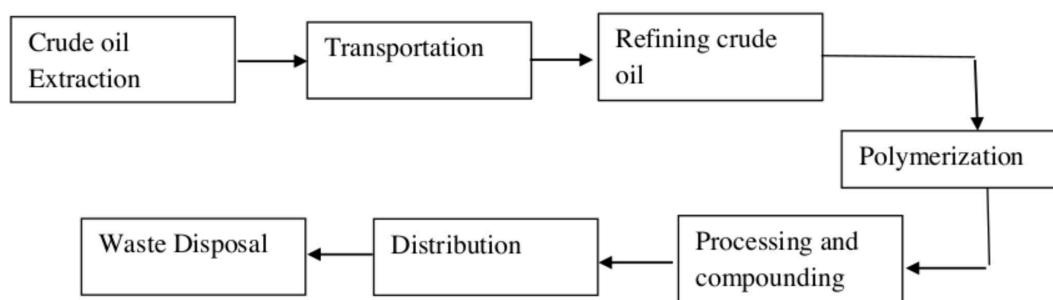


Figure 1.1 Flowchart for plastic production

1.1.4 Impacts of conventional plastics

- Effects of plastics on environment

Large amounts of plastic were found in dumps and outdoors. Plastic makes up about 10% of municipal trash discharge by weight, and it will be taken into consideration later. Newspaper reports of plastic trash on a few of the highest peaks demonstrate how leftover plastic can pollutes a wide variety of natural surface, freshwater, and aquatic ecosystems. There are further some statistics on littering in cities. There are reports of small pieces of plastic inadvertently polluting soils as outcome of transmitting sewage effluent, of glass or plastic fragments affecting compost made from municipal garbage, and of plastics debris acted upon waterways, streams, and eventually the sea by rain and flash floods. However, more inquiry on the amounts and consequences of plastic litter in natural ecosystems, farmland, and freshwater is clearly needed. As a result, most of the proof provided here is naturally from aquatic environments(Austin et al., 2018).

- Effects of plastics on marine environment

Many plastics end up in the water, whether they disappear on ground or at marine. Plastics are transported far away from their primary source by wind power, rivers, sewage systems, and ocean waves. Plastics dumped in landfills can still reach the marine ecosystem if they have not been properly handled, especially if they come from windblown wastes or uncontrolled dumps near the shore. Plastic items collect on sandy shores and ocean floors, including in wetlands, mangrove forests, submarine trenches, frozen seas, and barrier reefs. Plastic trash on shores and coastlines comes from both local resources especially wind and waves propelled items that have probably migrated millions of kilometres. Single-use products such as cigarette packets, wrappers of food, plastic bottles, the caps and lids, disposable bags, utensils, and shakers take-away boxes, and foam-like polystyrene boxes are among the most encountered plastic objects in international ocean clean-ups. Several of those things are also frequently found on sandy beaches in New Zealand. Currently, 344 faunas have been documented to be entangled, which includes 100% of aquatic

turtles, 31% of whales, 67% seals, 25% seagulls, 89 species aquarium fish, and 92 species of inverted vertebrates. Individuals that obtain plastics waste could be harmed by toxins connected to plastic products that are included during manufacturing or build up from polluted environmental matrices like soil or ocean. A minimum of 78% of the top pollutants designated by the US Environmental Protection Agency (EPA) have been established to be connected to polymer marine waste. Most of these compounds are Persistent, Bio accumulative, and even Toxic (PBT) (Thompson et al., 2009).

- Effects of plastics on human health

Plastics pose a risk to human wellness since they are used in healthcare processes and to safeguard our meals and drinks. Plastic vessels and bottles enable the distribution of safe-to-drink freshwater in areas where waterborne illness is a serious issue. Plastic wrapping prevents drinks and food from degradation due to microbial contamination. An increasing body of studies indicates that the continual accumulation of chemicals connected with plastics endangers our food's security and overall wellness. Microplastics were discovered in a variety of marine species, including fish as well as shellfish. It is further found in regular salt, well water, and up to 90% of H₂O that is packaged. Microplastics were recently found in the atmosphere and in household dust. From a daily basis, many people are likely inhaling in microplastics. Fortunately, it is unknown how much harm its exposure may cause humans or what happens when exposed to human beings.

- Effects of plastics on Animal

Many creatures, including reptilian species, mammals, vertebrates, and fish, could consume plastic waste when they mistake it as real prey and nutrition. The consequences can be hazardous with huge plastic pieces messing with an animal's ability to inhale, eat, or neutralize food, potentially leading in death. If plastics have been mistaken as meals and consumed, they may starve an organism, slow development, and impair the animal's ability to flee predators and breed. If microplastics are taken rather than food that can be digested, this results in

diminished feeding activity and even physical harm such lacerations and responses of inflammation(Dhar et al., 2018).

1.2 BIOPLASTICS

1.2.1 An Overview of Bioplastics

Bioplastics are plastics manufactured from renewable ingredients that include vegetable oils and fatty acids, corn starch, peas and beans starch and potato starch. It is seen as a greener choice to typical plastics derived from fossil fuels since they are both biodegradable and compostable, and they release greater greenhouse gases while processing. Bioplastics are receiving acceptance as a viable alternative to regular plastics in terms of environmental impact.

Bioplastics are an immersive changer in the sector of environmentally friendly substances. It is considered as a possible alternative to regular plastics due to growing concern about their environmental impact. In contrast to conventional plastics, that are generated from fossil fuels such as gasoline, bioplastics are created from renewable sources of biomass such as corn, sugarcane, and potato starch. One of the key advantages of bioplastics was their biodegradability(Austin et al., 2018).

This implies that bacteria can degrade them into natural chemicals like water, carbon dioxide, and biomass. As a result, they are compostable and have no impact to the build-up of plastic trash in garbage dumps and seas. Natural ingredients such as cellulose, starch and amino acids are commonly used to make biodegradable polymers. Enzymes, bacteria, and other microbes have the ability to degrade them.

The decreased carbon impact of bioplastics seems a further benefit. Traditional plastics are produced in large part using fossil fuels, that raises emissions of greenhouse gases and causes climate change. On the other hand, bioplastics are produced from sustainable sources resulting in a smaller carbon footprint. It can support sustainable development by lowering carbon emissions(Yin et al., 2013).

Bioplastics can be categorized in various ways depending on factors such as their sources, chemical compositions, production processes, and applications.

These definitions indicate some possible uses for bioplastics:

1. biodegradable and based on renewable resources;
2. although not being biodegradable, based on renewable resources;
3. based on fossil fuels and biodegradable

The two main types of bioplastics are biodegradable and non-biodegradable. Sustainable bioplastics are made to degrade under particular circumstances, like extreme heat or contact with particular microorganisms. The term "bio-based plastics," is also referred to as "non-biodegradable bioplastics," refers to plastics manufactured from renewable resources but which do not decompose. Figure 1.1 shows the classification of bioplastics. Both recycling and the production of new items can be done with these resources (Gironi & Piemonte, 2011).

- Advantages of bioplastic

Bioplastics have unique properties such as being beneficial to the environment, reusable and compostable, biodegradable, and energy efficient. The prospects for the use of biodegradable plastics are bright. Following list a few of the benefits of bioplastics:

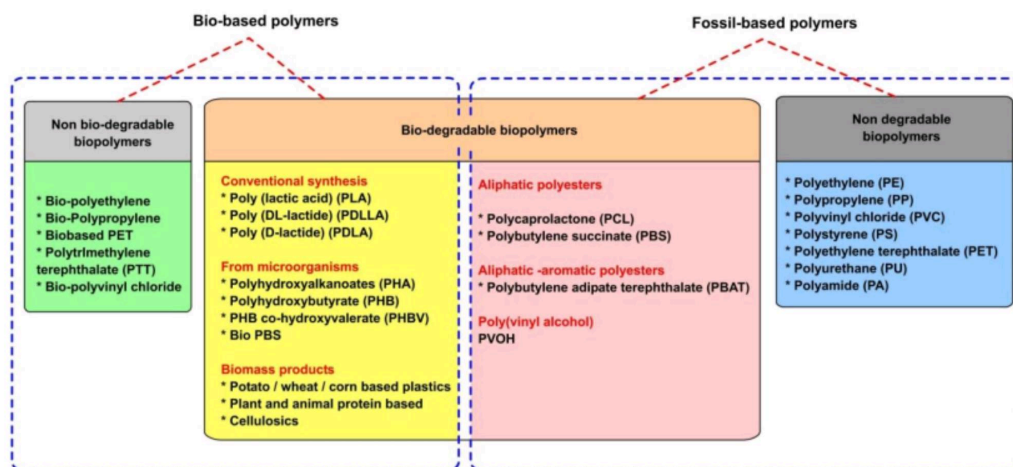


Fig.1.2; Classification of bioplastic

1. Renewable: Bioplastics can be created in an effective way because they are generated from renewable materials.
 2. Biodegradable: Some varieties of bioplastics are capable of decomposing harmlessly and biologically in the environment.
 3. Versatility: There are many applications for bioplastics, ranging from disposable items and packaging to car parts and surgical devices (Atiweh et al., 2021).
 4. Reduced carbon footprint: When bio-based plastics made from green energy sources, bioplastics could have a smaller carbon impact than conventional plastics.
 5. Reduced reliance on fossil fuels: Bioplastics can aid in reducing our reliance on finite, not sustainable fossil fuels.
- Disadvantages of Bioplastics

However, issues could arise while using bioplastics. The following are a few drawbacks of bioplastics:

1. Land usage: The crops required to manufacture bioplastics must be grown on land, which may impose pressure on habitats and agricultural practices.

2. Production concerns: Producing bioplastics may prove more expensive and labour-intensive than producing conventional plastics.
3. Misuse: Bioplastics can occasionally be confused for conventional plastics, that are result in them being placed in recycling systems where they are not intended, causing contamination, and lowering recycling rates.
4. Energy consumption: To make bioplastics, energy is needed, some of which may be derived from non-renewable resources that increase greenhouse gas emissions(Song et al., 2011).
5. Limited recycling option: There are not many options for recycling for bioplastics, even though some of them can be recycled. As a result, they might end up in incineration or landfills or in certain cases, they may be burned.

1.2.2 Bioplastics as a closed loop approach

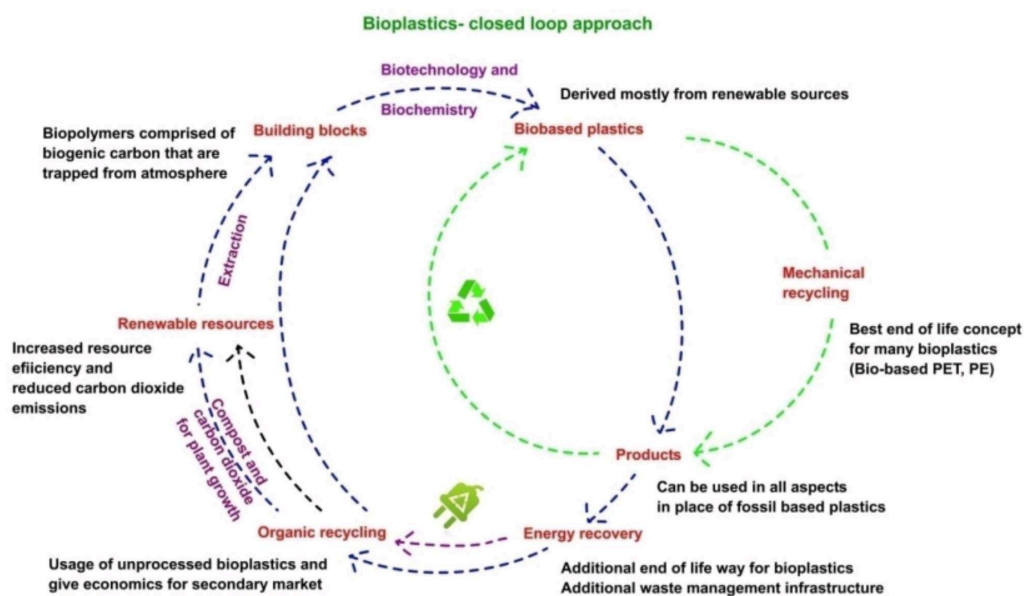


Fig.1.3; Life cycle of Bioplastic

Because they are made from renewable resources and can be composted, bioplastics can be regarded as a closed-loop strategy. Traditional plastics are not

biodegradable and are made from petroleum, an unrennewable resource, which causes pollution and harm to the environment.

On the contrary, sources of renewable energy that can be repeatedly cultivated and extracted include cellulose, sugarcane, and maize starch, which are used to make bioplastics. Additionally, bioplastics can be composted, where they decompose into organic material that can be applied as fertiliser naturally. The cycle is then continued by using the resulting compost to grow additional crops(Al-Salem et al., 2009).

Additionally, some bioplastics have the ability to be recycled, resulting in a closed-loop system where the materials can be used repeatedly before being composted at the end of their useful lives. As a result, fewer new resources are required, and less waste ends up in landfills and the oceans.

Figure 1.3 says bioplastics provide a more environmentally responsible and sustainable option to conventional plastics, offering a closed-loop system that encourages the effective use of natural resources and reduces waste(Grigore, 2017).

1.2.3 Current state of Bioplastics in India

Bioplastics have grown popularity in recent times because of their sustainability and biodegradability. The biodegradable plastics business in India is quickly expanding, with a yearly expansion rate around 30% estimated to hit a worth of USD 1.7 billion by 2025. This rise in consumption can be linked to people being more conscious of the adverse impacts of conventional plastics on the surroundings, as well as government aims to encourage sustainable practises. Several Indian companies have been producing bioplastics from sustainable resources including corn starch, sugarcane, and vegetable oil lately. These plant-based plastic are not solely biodegradable, but they also exhibit a reduced carbon impact than regular plastics. For example, Natur-Tec, makes bioplastics from a combination of corn and starch from potatoes. The organization's natural plastics are utilized in a variety of applications, includes packing, farming, and consumer products.

Another business, Bioplast India, produced a cornflour-based recyclable plastic. The items sold by the business have a life span of six months and may be used for a variety of things, including grocery bags, wrapping food, and temporary flatware. The business has also created a selection of recyclable plastic bags that may be used in place of conventional reusable bags (Sidek et al., 2019).

To encourage the usage of bio-based plastics in the nation, the Indian legislature has also launched several initiatives. Its announcement of a restriction on single-use plastics including drinking straws, utensils, and baggage in 2018 has spurred demand for sustainable substitutes. In order to promote the manufacturing of bio-based plastics in the nation, the government of India has also introduced a few programmes. In India, yet, there remains some issues required to be resolved despite the increasing prevalence of bioplastics. The price of biodegradable plastic is considerable compared to conventional plastics, which serves as one of the main obstacles. Due to their higher production costs, bioplastics are now less available for the consumers. Another issue that must be solved is the absence of construction for the disposal and reuse of bioplastics.

India's bioplastics business is expanding quickly, and it seems there is more opportunity for it to take a significant position in the worldwide bioplastics business. In the upcoming years, the need for reusable alternatives is anticipated to rise as an outcome of federal efforts and growing public awareness of the negative environmental consequences of conventional plastics. The expense and the absence of infrastructure for waste disposal and recycling are two issues that must yet be resolved (Rosenboom et al., 2022).

1.2.4 Biodegradation of bioplastics

The ability of bioplastics to break down much more quickly than conventional plastics is one of their primary benefits. Depending on the surroundings and the type of bioplastic, some bioplastics can biodegrade in a matter of months, whereas conventional plastics can take many generations to break down. Bioplastics may

degrade by means of enzymatic breakdown, hydrolysis, and oxidation, among other processes.

Bioplastics are broken down into simpler substances by enzymes through a process known as enzymatic degradation. Natural catalysts for chemical processes, or enzymes are materials formed by microorganisms like bacteria and fungi. Water and soil are two environments where enzymatic degradation can happen (Emadian et al., 2017).

Water breaks down bioplastics through a process known as hydrolysis. When exposed to both moisture and heat, bioplastics can undergo a process called hydrolysis in which the chains of polymer are broken down into simpler compounds. Compost and anaerobic digestion are two environments where hydrolysis can happen.

By being exposed to oxygen, bioplastics undergo a process known as oxidation. When bioplastics are exposed to air and UV light, they can oxidize, disintegrating into more basic compounds. Soil and water are two different environments where oxidation can happen.

The environmental advantages of bioplastics' biodegradation are numerous. Microplastics or dangerous chemicals are not left behind when bioplastics degrade. Water and carbon dioxide are natural byproducts of biodegradation that don't harm the environment (Russell et al., 2011).

The environmental effects of plastic contamination can be lessened by reducing the amount of bioplastic waste that ends up in landfills and oceans.

A promising area of study with the potential to lessen the environmental harm caused by plastic waste is the biological degradation of bioplastics. Bioplastics may breakdown much more quickly than conventional plastics in a variety of environments, such as soil, water, and compost on it. The potential of bioplastics to degrade in any environment and their emission of greenhouse gases while degrading are two issues that are connected to the biological degradation of bioplastics, though.

As research advances, it will be essential for reducing greenhouse gas emissions to develop bioplastics that can break down in all environments(Shavandi et al., 2017a).

- Factors affecting biodegradation

The kind of bioplastic is one of the most important factors affecting biological degradation of bio plastics. Each of the materials used to make bioplastics, including starch, cellulose, and PLA, has specific characteristics that have an impact on how quickly the material degrades. For instance, bioplastics made of starch degrade readily in compost but may not do so as well in soil or water. Contrarily, PLA may degrade in a variety of environments, such as compost, soil, and water, but they call for particular circumstances, like high temperatures and the presence of particular microorganisms.

1. Environment is the another element influencing the natural breakdown of bioplastics. Although biodegradation of bioplastics can occur in a variety of environments, including soil, water, and compost, the conditions needed for biological degradation can differ based on the environment. Bioplastics that breakdown in soil, for instance, might need different circumstances than those that degrade in water. The environment's moisture, temperature, and amount of oxygen can also have an impact on how quickly things degrade(Prasetyo et al., 2017a).
2. The breakdown of bioplastics is also influenced by their size and shape. Microorganisms can more easily access bioplastics that are minimal and have a lot of surface area, which can hasten the biodegradation of those materials. However, large or complex-shaped bioplastics might be more challenging for microbes to access, which could impede biodegradation(Folino et al., 2020).
3. Last but not least, the existence of additives in bioplastics may also influence how well they degrade. Some additives, like plasticizers and stabilizers, can stop microorganisms from rupturing the polymeric chains in bio plastics, thereby inhibiting the biodegradation of those materials. By encouraging oxidation and disintegration, other additives like pro-oxidant compounds and

photodegradable agents can hasten the breakdown of bioplastics(Harmaen et al., 2015).

1.2.5 Sources of biomass

- ²¹ Cellulose

Cellulose is a highly abundant organic polymer on our planet and it acts as a straight homo-polymer of sucrose. In contrast to the structure in starch, a glucose repeated units are connected in this configuration. This permits the links to crystallize in a straight path as highly crystallized, compact, sub-micron the measurement micro-fibrils arranged along the cellulose fibre. Vegetable fiber, with certain exceptions such as cotton fibers, which is practically pure cellulose, existing as an organic bio-composite contains cellulose particles held together by hemicelluloses and the lignin substance found in plant cell walls to provide structural support. According to the degree of modification, there are several categories for direct cellulose use in wrapping, that range due to microfibrils, log pulp (small fibre), textile materials, or fibre optic technology, to comparatively untreated lignocellulosic biomass like wooden flour, drinking straws etc. Additionally, by altering the chemical composition of cellulosic derivatives, regenerated cellulose film (RGF) could be created(Camberos-Luna et al., 2016).

- Chitin

³⁵ It is the second most widespread natural polymer on Earth, after cellulose. Chitosan originates from chitin. Chitosan actually a byproduct of the fishing industry that is produced through a breakdown process in an existence of alkali. It creates films without the use of chemicals and has superior mechanical and microbiological qualities against molds, microbes, and yeasts. This also has strong oxygen and dioxide flexibility, but the main disadvantage is the fact it is poorly soluble in neutral environments. The needed degree of breakdown to generate a dissolved product is 80-85%. It generates translucent films, which increases the product's value and storage life.

- Starch

Starch acts as a plentiful, inexpensive, and environmentally friendly form for organic polymer, making it a very desirable raw material for the production of bioplastics. The starchy substance is the carbohydrates copolymer of D-glucose that is found as granular particles in many crops, including corn, grain, rice, tapioca, & potatoes. varied sources of starch have varied ratios of different ingredients, molecular weights, varieties and levels of crystallinity, and granule sizes, which result in variations in characteristics. Because of its elevated temperature and brittleness, organic starch is unsuitable for use as a biodegradable plastic and needs to be reconfigured and plasticized while surrounded by of water using temperatures mechanical shear, when the addition of plasticizers such as glycerol and polyols. Compounded starch is used to bridge distinct polymer chains either with or without the use of bioplastics., produces an array of polymers known as starch complexes(Ezeoha, 2013).

- Proteins

Enzymes may break down proteins, which are organic chains of amines connected by amide bonds. Protein-based materials have been utilised as encapsulates, protective coatings, sticks, and cleansers since the 1930s. animal products and plant-derived proteins may be distinguished based on where they come from(Chilakamarry et al., 2021). Manufacturing food-safe containers is particularly appealing due to the nutritious benefits of proteins. Materials comprised of proteins coverings and films as well as edible film and coatings can be available with detailed explanations.

1. Animal base proteins: An important family of amino acids found in the extracellular layer of animal tissues is collagen. (bones, tendons, and skin). Collagen, which is decomposed by high-alkaline gives gelatine, it has been employed in food preparation and medication encapsulation. Plasticization, such as glycerin or mixing with different polymers, such as PVOH, might modify the physical and chemical characteristics(Sharma et al., 2018).

2. Plant based proteins: The beans (US) or soy (UK) has 38% amino and 18% oil, from other things. These were used to make polymers from soy. Soybean protein and oil are easily utilised to make sustainable highly rigid thermoset polymers, such as Soyad, which can substitute formaldehyde and urea glue in the fabrication of wood fibres board.

1.3 KERATIN

1.3.1 A Summary of Keratin

Keratin has increased in prominence over the past few years as a result of its remarkable biocompatibility and biodegradability. It has demonstrated promising outcomes in a variety of industries, including poultry, textiles, agriculture, personal care products and pharmaceuticals. Keratin acts as a multifunctional biopolymer which has been employed in the manufacturing of fibrous materials, and it may be converted into gel forms, films, nanoparticles, and microparticles with some necessary modifications. Its resistance to enzymatic breakdown and exceptional biocompatibility leading to applications for biomedicine and medical regeneration(Pourjavaheri et al., 2019a).

Keratin-based bioplastics are a form of bioplastic generated from a substance called keratin, the fibrous protein that occurs in hair, feathers, claws, and horns. It has been utilised in a variety of purposes, includes beauty products, healthcare and meals. Keratin-based biodegradable plastic have various advantages over standard petroleum-based plastics, which includes biodegradability, sustainability, and the ability for minimizing waste.

- Benefits Of Using Keratin Based Bioplastics

In this essay, we will look at the advantages of keratin-based bioplastics and why they can serve as a promising alternative to regular plastics.

1. Ecological Sustainability:Keratin-based bioplastics are regarded as environmentally friendly because they are created from a widely available

natural substance. Bioplastics are thought to be kinder to the environment than typical plastics derived from non-renewable resources that include oil. This is due to the fact that the manufacture of traditional plastics emits greenhouse gases, but the manufacture of bioplastics emits lower greenhouse gas emissions in general.

2. Renewable: Keratin is a renewable resource since it may be derived from a range of animal by-products, including hair and wings. As a result, keratin-based bioplastics are a more sustainable and greener alternative to petroleum-based plastics(Shavandi et al., 2017b).
3. Waste reduction: The usage of keratin-based bioplastics may help to reduce wastage by reusing otherwise discarded animal by-products. This has the potential to benefit both the surroundings and the local economy by reducing trash and providing a potential source of revenue for agriculture and other enterprises.
4. Strength and durability: Keratin is a tough and durable substance, making it a great choice for usage in a wide range of applications such as packaging and textiles(Pourjavaheri et al., 2019b; Ramakrishnan et al., 2018).
5. Flexibility: Depending on the intended usage, keratin-based bioplastics can be adjusted to fulfil certain specifications and needs, like flexibility or hardness.
6. Water resistance: Keratin-based bioplastics have been demonstrated to be extremely resistant to water, which makes them perfect for usage in applications such as wrapping food.
7. Low toxicity: Keratin is a natural material that is widely regarded as non-toxic, decreasing the possibility of harmful environmental or health effects linked with its use.
8. Potential for medical applications: Due to their strength and biocompatibility, keratin-based bioplastics have the ability to be used in medical products such as sutures.

9. Antibacterial characteristics: Some study suggests that keratin-based bioplastics may have antibacterial qualities, which could be valuable in medical applications or in decreasing food waste.
10. Challenges with sourcing: the keratin is a renewable resource, there are some challenges with finding and preparing it for usage in bioplastics. The grade and purity of the keratin can vary based on its source and processing methods utilized, which might have an effect on the qualities of the final bioplastic.
11. Cost: Due to the processing and purification required, the expense of generating keratin-based bioplastics may be higher than ordinary plastics. However, as manufacturing facilities and technology enhance the cost of these materials is projected to fall (Saharan & Sharma, 2015).
12. Limited availability: While keratin is renewable in nature, its accessibility and consistency can be affected by the source or processing methods used.
13. Potential for allergenicity: Keratin is a protein, and some people may be allergic to it. More research is required to determine the allergenic effects of keratin-based bioplastics.
14. Applications in packaging: Due to their rigidity and water resilience, keratin-based bioplastics have the ability to be utilised in a variety of packaging sectors, such as shipping supplies and food packing.
15. Textile applications: Due to their strength and durability, keratin-based bioplastics can be used for the manufacturing of textiles like as clothes and upholstery (Mohapatra, A., Prasad, S., Sharma, 2017).

1.3.2 Human hair as a source of keratin extraction

Keratin-based bioplastics are a viable alternative to standard petrochemical-based plastics since they are biodegradable, reusable, and non-toxic. Bioplastics can be made from a range of natural materials, including human hair. The main component of hair is keratin, a protein with fibrous structure that gives hair, nails, and skin structural strength. Keratin is a naturally occurring polymer with distinct features that

make it an excellent choice for bioplastic manufacturing. It is non-toxic, biodegradable, and has outstanding mechanical qualities such as high tensile strength, flexibility, and rigidity(Chilakamarry et al., 2021).

The easy availability and abundance of human hair as a raw material for keratin-based bioplastics is one of its key benefits. Hair is a natural resource that can be recycled and it is frequently generated by humans, which makes it a sustainable and environmentally benign option for bioplastic manufacture. Furthermore, because hair is frequently wasted as a consequence of cosmetics industry, reusing it as a raw material can help to decrease waste and pollution. Human hair has another benefit in that it is inexpensive and can be used to make bioplastics. In comparison to other keratin sources, like feathers or hooves, human hair is more affordable because it is readily available and easy to get. Hair is a versatile and readily accessible raw material because it may be procured in a variety of places, such as barber shops, salons, and even animal shelters(Maulida et al., 2016).

There are certain drawbacks to using human hair as a raw material, notwithstanding its long-term viability as a source for keratin-based bioplastics. One of the major issues is the variety in hair quality and composition, which might impact the qualities of the final bioplastic. For instance, chemically altered or damaged hair may result in a weaker or inferior bioplastic. Overall, human hair offers a promising source for keratin-based bioplastic films. It is a desirable alternative for producing sustainable bioplastics because of its availability, quantity, and affordability. To assure the creation of high-quality bioplastics, it is important to take carefully into account, the variations in hair content and quality as well as the difficulties in processing. Human hair-based bioplastics have the ability to replace conventional petrochemical-based plastics as a viable alternative with more research and growth(Thomas et al., 2012)

CHAPTER-2

LITERATURE SURVEY

- **Gomez-Martinez et al. (2011)** had published the journal in the topic of "Modeling of Pyrolysis and combustion of gluten–glycerol-based bioplastics". Wheat gluten and gluten-based bioplastics' thermal degradation has been studied using non-isothermal thermo-gravimetric analysis in nitrogen and air atmospheres. Researchers prepared bioplastic from gluten and glycerol. They analyze the bioplastic by thermogravimetric evaluation, Thermal deterioration and the thermogravimetric model of gluten–glycerol-based bioplastics. Models based on pseudo components that correspond to the primary gluten isolate and bioplastic molecules, i.e., water, glycerol, starch, glutenin, and gliadin, have been used to simulate the thermal degradations of gluten and glycerol-gluten-based bioplastics. The simulation shows that the protein matrix and bioplastic moisture content both influence and impede glycerol volatilization.
- **M. Li et al. (2013)** extracted keratin from human hair using reducing agents and prepared bioplastics with high transparency and good mechanical properties. The researchers extracted keratin from human hair using a mixture of reducing agents, and used the extracted keratin to prepare bioplastics films. The bioplastics films exhibited high transparency and good mechanical properties. The results of plasticizer type and focus on the characteristics films made from bioplastic was also studied by the researchers.
- **J. Li et al. (2014)** created bioplastics from polyvinyl alcohol and human hair keratin, producing films with exceptional transparency and advantageous mechanical characteristics. In this work, keratin from human hair was isolated and polyvinyl alcohol, a biodegradable and water-soluble polymer, was used to create bioplastics. The solution casting technique was used by the researchers to create bioplastics, and they then examined the films' mechanical, thermal, and optical characteristics. The outcomes demonstrated that the bioplastics exhibited strong mechanical characteristics, for example, tensile strength,

modulus, and lengthening at break, as well as great transparency. The features were attributed by the researchers to interactions between the molecules of polyvinyl alcohol and keratin.

- **H. Shang et al. (2014)** synthesized bioplastics from keratin extracted from human hair and polyethylene glycol, resulting in films with high transparency and good mechanical properties. to create bioplastics using water-soluble and biocompatible polymer polyethylene glycol with human hair keratin that has been removed. The solution casting technique was used by the researchers to create bioplastics, and they then examined the films' mechanical, thermal, and optical characteristics. The outcomes demonstrated that the bioplastics exhibited strong mechanical characteristics, such as tensile strength, modulus, and elongation at break, as well as great transparency. The capacity of the plasticizer to improve the intermolecular connections between the keratin molecules is what the researchers said was responsible for the qualities.
- **T. M. Y. S. Tawakkal et al. (2015)** synthesized bioplastics from keratin extracted from human hair and polycaprolactone, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and polycaprolactone, a biodegradable and biocompatible polymer. The researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, Regarding the films' optical characteristics. The outcomes demonstrated that the bioplastics exhibited strong mechanical characteristics, such as tensile strength, modulus, and elongation at break, as well as great transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and polycaprolactone.
- **M. A. Rehman et al. (2015)** synthesized bioplastics from keratin extracted from human hair and polyethylene glycol, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and polyethylene glycol, a water-soluble and biocompatible polymer. The researchers prepared bioplastics using a solution casting method and tested the mechanical,

thermal, and the films' optical characteristics. The findings revealed that the bioplastics had high transparency and good mechanical attributes, including Tensile power, modulus, and lengthening at the break. The researchers attributed the properties to the plasticizer's ability to enhance the intermolecular interactions between the keratin molecules.

- **S. S. Jahan et al. (2015)** prepared bioplastics from keratin extracted from human hair and polyvinyl alcohol, resulting in films with high transparency and good mechanical properties. To prepare bioplastics from keratin extracted from human hair and polyvinyl alcohol, a biodegradable and water-soluble polymer. The researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, and optical qualities of the movies. According to the findings, the bioplastics possessed excellent mechanical qualities, including strong tensile strength, modulus, and elongation at break. The researchers attributed the properties to the intermolecular interactions between the keratin and polyvinyl alcohol.
- **N. H. Thanh et al. (2016)** synthesized bioplastics from keratin extracted from human hair and polycaprolactone, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and polycaprolactone, a biodegradable and biocompatible polymer. The solution casting technique was used by the researchers to create bioplastics, and they then examined the films' mechanical, thermal, and optical characteristics. The outcomes demonstrated that the bioplastics exhibited good mechanical attributes, such as modulus, elongation at break, and tensile strength, as well as great transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and polycaprolactone.
- **M. M. Yusuf et al. (2016)** prepared bioplastics from keratin extracted from human hair and glycerol, resulting in films with high transparency and good mechanical properties. To prepare bioplastics from keratin extracted from human hair and glycerol, a natural plasticizer. The researchers prepared bioplastics using a solution casting method and tested the mechanical,

thermal, and optical characteristics of the films. The outcomes indicated that the bioplastics had high transparency and good mechanical characteristics, including elastic modulus, modulus, as well as elongation at break. The researchers attributed the properties to the plasticizer's ability to enhance the intermolecular interactions between the keratin molecules.

- **M. A. Al-Ghouti et al. (2017)** investigated the extraction of keratin from human hair using various reducing agents and characterized the extracted keratin using FTIR, XRD, and SEM. The study found that sodium sulfite and sodium hydroxide were effective in extracting keratin from human hair, and the extracted keratin exhibited a high degree of purity.
- **K. R. Rajendran et al. (2017)** used different solvents and plasticizers to prepare bioplastic films from human hair keratin with high transparency and biodegradability. This study explored the use of different solvents and plasticizers to prepare bioplastic films from keratin extracted from human hair. The study found that the resulting bioplastic films had high transparency and biodegradability, making them promising candidates for eco-friendly packaging materials.
- **R. T. Pawar et al. (2017)** investigated the outcome of plasticizer type and focus on the characteristics of bioplastics made from keratin in human hair. The researchers synthesized bioplastics films from keratin extracted from human hair, and examined the effect of different plasticizers (glycerol, sorbitol, and polyethylene glycol) on the properties of the bioplastic's films. They found that the type and concentration of plasticizer had a considerable impact on the films made of the bioplastic's mechanical and thermal properties.
- **X. Wang et al. (2018)** compared the properties of bioplastics from human hair and feather keratin, finding that human hair bioplastics had higher transparency and mechanical properties and prepared bioplastics films from keratin extracted from both human hair and feathers, and compared the properties of the resulting bioplastics films. They found that the human hair bioplastics films exhibited higher transparency and better mechanical properties than the feather bioplastics films.

- **W. Zhang et al. (2019)** investigated that bioplastic films using keratin extracted from human hair and characterized the films using FTIR, XRD, SEM, and tensile strength testing. The study found that by including sorbitol as a plasticizer improved the mechanical qualities, as well as great transparency and strong tensile properties.
- **S. Gómez-Meléndez et al. (2019)** prepared bioplastic films using keratin extracted from human hair and characterized the films using FTIR, XRD, and SEM. The study found that the addition of glycerol as a plasticizer improved the flexibility of the films, and the films exhibited good mechanical properties.
- **Zhang et al. (2019)** investigated the use of human hair keratin as an energy source for the preparation of transparent bioplastics. Using a variety of reducing agents, the researchers isolated keratin from human hair, and the collected keratin was then dissolved in acetic acid to create a solution. Afterwards, a casting machine was used to cast the solution into films, which were subsequently tested for tensile strength, thermal stability, and water resistance. The findings demonstrated that the bioplastic sheets exhibited excellent mechanical qualities, high water resistance, and great transparency.
- **J. Wu et al. (2019)** prepared bioplastics from human hair keratin using a natural plasticizer, citric acid, resulting in bioplastics with high transparency and mechanical strength. The researchers used citric acid as a natural plasticizer to prepare bioplastics films from keratin extracted from human hair. The bioplastics films exhibited high transparency and mechanical strength, and the researchers found with the inclusion of citric acid improved the thermal stability of the bioplastics films.
- **S. Oikonomou et al. (2019)** produced bioplastics from human hair keratin and a biodegradable plasticizer, glycerol, resulting in transparent and flexible films. Synthesized bioplastics films from keratin extracted from human hair and a biodegradable plasticizer, glycerol. The resulting bioplastics films were transparent and flexible, and the researchers found that The inclusion of glycerol increased the films' mechanical characteristics.

- **H. S. El-Sabbagh et al. (2020)** used a mixture of glycerol and urea as plasticizers to prepare bioplastics from human hair keratin with high transparency and mechanical strength. Researchers investigated the use of a mixture of glycerol and urea as plasticizers in the preparation of bioplastics from keratin extracted from human hair. The study found that the resulting bioplastics had high transparency and mechanical strength, indicating the potential of the glycerol-urea mixture as a plasticizer for human hair bioplastics.
- **Gupta et al. (2020)** research included a project to create bioplastic films utilising keratin taken from human hair. The keratin was isolated from human hair by the researchers using a variety of reducing agents, and the retrieved keratin was then dissolved in formic acid to create a solution. Using a spin coating process, the solution was cast into films, and the films' thermal stability, mechanical characteristics, and water vapour permeability were all evaluated. The outcomes demonstrated that the bioplastic films had good mechanical and thermal durability as well as high transparency and low water vapour permeability.
- **Pramanik et al. (2021)** explored the use of human hair keratin in the synthesis of bioplastic films for food packaging applications. Using sodium sulfite and sodium hydroxide, the researchers isolated keratin from human hair, and the collected keratin was then dissolved in formic acid to create a solution. Using a casting machine, the solution was transformed into films, and the films' mechanical characteristics, water vapour permeability, and thermal stability were assessed. The findings demonstrated that the bioplastic sheets were acceptable for food packaging applications due to their superior mechanical capabilities, low water vapour permeability, and strong thermal stability.
- **A. Rahman et al. (2021)** prepared bioplastics from keratin extracted from human hair and chitosan, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and chitosan, a biodegradable and antimicrobial polymer. The researchers prepared bioplastics using a solution casting method

and tested the mechanical, thermal, and optical film's characteristics. According to the findings, the bioplastics exhibited superior mechanical qualities, including good tensile strength, modulus, and elongation at break, and they also had a high degree of transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and chitosan.

- **F. Zhang et al. (2021)** synthesized bioplastics from keratin extracted from human hair and modified starch, resulting in films with high transparency and biodegradability. To prepare bioplastics from keratin extracted from human hair and modified starch, a renewable and biodegradable material. The researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, and optical a film's characteristics. According to the findings the bioplastics had high transparency and biodegradability, with the degradation rate increasing with increasing starch content.
- **R. Natarajan et al. (2021)** synthesized bioplastics from keratin extracted from human hair and polyvinyl alcohol, resulting in films with high transparency and good mechanical properties. bioplastics from keratin extracted from human hair and polyvinyl alcohol, a biodegradable and water-soluble polymer. The scientists created bioplastics by solution casting them, and then they evaluated the films' mechanical, thermal, and optical characteristics. According to the findings, the bioplastics exhibited good mechanical properties, including good tensile strength, modulus, and elongation at break, and they also had a high degree of transparency. The researchers attributed the properties to the intermolecular interactions between the keratin and polyvinyl alcohol.
- **Y. Liu et al. (2021)** prepared bioplastics from keratin extracted from human hair and citric acid, resulting in films with high transparency and good mechanical properties. This study aimed to prepare bioplastics from keratin extracted from human hair and citric acid, a natural plasticizer and researchers prepared bioplastics using a solution casting method and tested the mechanical, thermal, and the films' optical qualities. The findings revealed that

the bioplastics exhibited excellent mechanical properties, including high transparency and strong tensile strength, modulus, and elongation at break. The researchers attributed the properties to the plasticizer's ability to enhance the intermolecular interactions between the keratin molecules.

¹ CHAPTER-3

AIM & SCOPE

3.1 AIM

- The purpose of this project is to investigate if it is feasible to create functional protein Biofilms from human hair debris.
- Our study's goal is to investigate the keratin-based bioplastic films' characterisation, optimization, and prospective applications. We shall test the antibacterial activity of biofilm in this study.
- This research is based on the "Synthesis and characterisation of keratin based bioplastic film using human hair" riches from waste principle.

3.2 SCOPE

- In biological applications and in the food packaging industries
- Raw materials for various hygiene goods, such as nappy products' superabsorbent materials
- In agricultural sectors and tissue engineering (e.g., replacement of artificial hip joints, artificial skin and hearts).
- Used in the fashion sector as artificial skin replacement and breast implants.
- In the pharmaceutical sector (e.g., transdermal drug delivery systems, wound dressing, and medication delivery).
- It is utilised in composite materials such as reinforcement for building materials and superconducting system composites.

CHAPTER-4

MATERIALS AND METHODS

4.1 OVERALL PROCESS

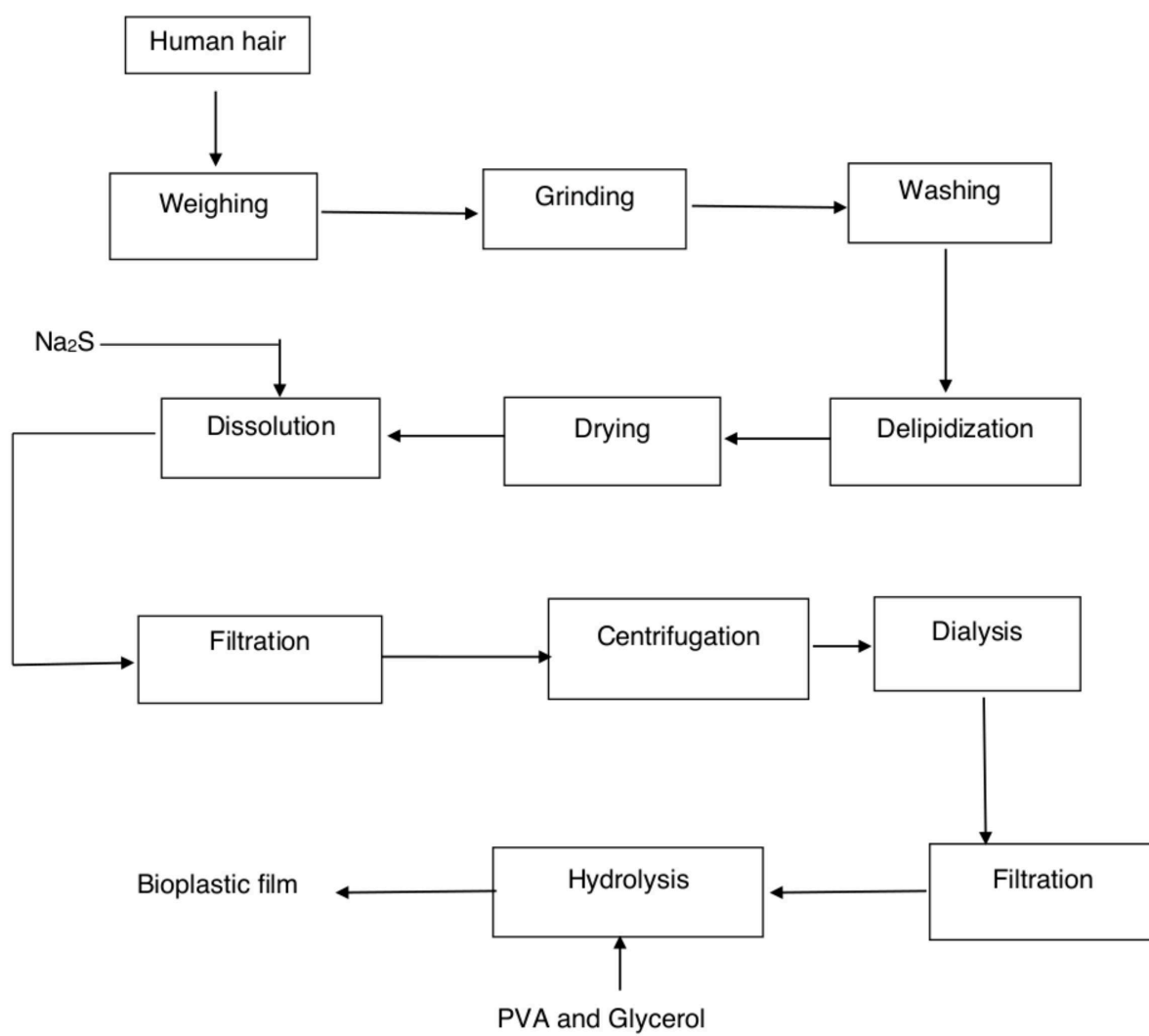


Fig.4.1; Overall process of keratin-based bioplastic film production

4.2 SELECTION OF MATERIALS

The unwanted human hairs were gathered from a nearby salon in OMR, Chennai, Tamil Nadu. Chemicals from Mercury Chemicals, Salem, included sodium sulfide, sodium hydroxide, ethanol, chloroform, glycerol, copper(II) sulphate, and benzoic acid, an antibacterial agent. The solution and washing were made with the distilled water.

Table 4.1: Usage of chemicals

S.No	Chemical used	Manufacturer
1	Ethanol	NICE chemicals
2	Chloroform	NICE chemicals
3	Glycerol	NICE chemicals
4	Sodium sulfide	NICE chemicals
5	Copper(II) sulphate	NICE chemicals
6	Benzoic acid	NICE chemicals
7	Polyvinyl alcohol	NICE chemicals
8	Sodium hydroxide	NICE chemicals
9	Distilled water	NICE chemicals

4.3 PRE-TREATMENT OF HUMAN HAIR

Before the keratin was taken out from the human hair, a few steps were taken. The following steps were part of the pre-treatment process.

- Weighing
- Grinding
- Washing
- Delipidization
- Drying

4.3.1 Weighing

First, the used human hairs were weighed. In the process of extraction, human hair with a total weight of about 50gm was used. The difference in weighing before and after washing was used to calculate the ratio of impurities to human hair.

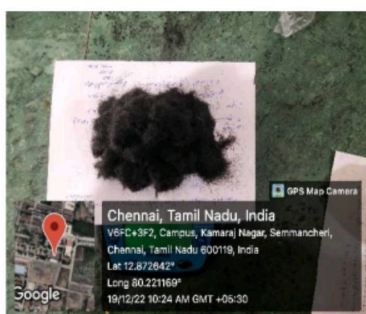


Fig.4.2;a)Human hair weighing



b)Human hair cutting

4.3.2 Grinding

Using a grinder, the provided human hairs were cut into tiny (0.2–0.3 cm) pieces. The unwanted materials, such as stones, papers, etc., were taken out before the grinding process.

4.3.3 Washing

Before using them to make plastic, the human hairs were thoroughly cleaned with soap and distilled water to get rid of any impurities like dirt and other unwanted materials. The human hairs were first cleaned, and then ethanol was applied to kill any remaining microorganisms. Proper washing was carried out to prevent contamination of the finished product. The cleaned hairs were then dried outside in the sun for 24 hours.

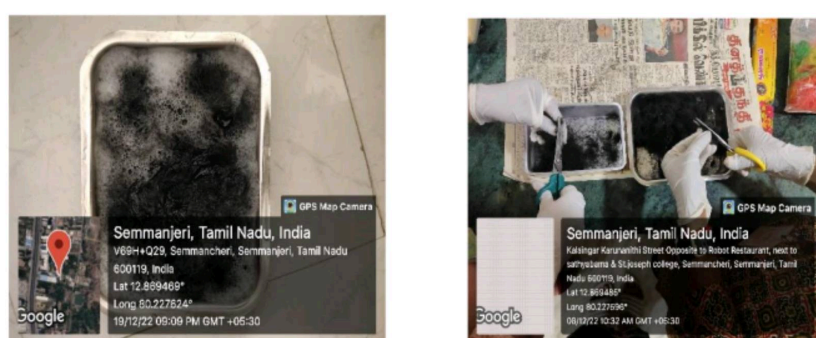


Fig.4.3; Human hair washing with soap

4.3.4 Delipidization and drying

The process of delipidizing involves removing lipids or lipid groups from a given substance. After being delipidized (1:2, v/v) with ethanol and chloroform for 24 hours at room temperature to remove the lipids from the hair, the hairs were then dried in a hot oven before the protein extraction step as in figure 4.4. To delipidize human hair in an industrial setting, lipid extractors and flash dryers will be used.



Fig.4.4; a) Ethanol and delipidization process b)Drying of treated hair

4.4 KERATIN EXTRACTION FROM HUMAN HAIR

There are several methods for extracting keratin using human hair, including the The sodium sulphide approach, the peracetic acid method, the thioglycolic acid method, and the urea method. We use the sodium sulphide method for extracting keratin because it is an easy and affordable process. This extraction process includes various processes such as dissolution, filtration, centrifugation, dialysis, and so on.

4.4.1 Dissolution of human hair with sodium sulfide

A total of 25 g of cleaned, dried, and delipidized hairs were added to one litre of a (100, 300, and 500 mM) sodium sulphide solution as shown in table 4.2 and heated at 50–60°C for 5–6 hours while being continuously stirred with a magnetic Stirrer. Nearly all human hairs have been dissolved after 6 hours, and the remaining hairs were separated by a filtration process.

Table 4.2:Concentration of sodium sulfide

Weight of human hair (g)	Concentration of sodium sulfide (mM)	Weight of sodium sulfide (g)
25	100	5.49
25	300	16.49
25	500	27.49

4.4.2 Filtration process of dissolved hair

The dissolved solution was filtered by running it through whatman filter paper. The retentate were eliminated following the filtration procedure.

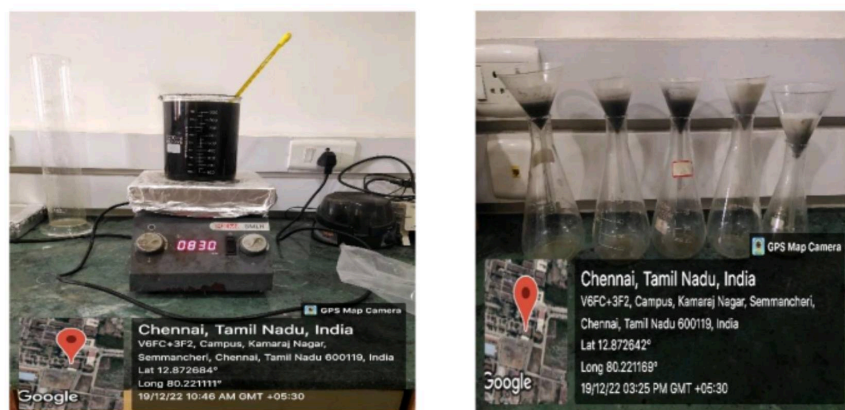


Fig.4.5; a) Dissolution of hair

b)Filtration of human hair

4.4.3 Centrifugation of filtrate

Following filtration, the filtrate solution underwent a 20-minutes centrifugation at 5000 rpm. The particles contained in the supernatant were removed and discarded. Hydrochloric acid solution was used to bring the supernatant's pH to 3.5.

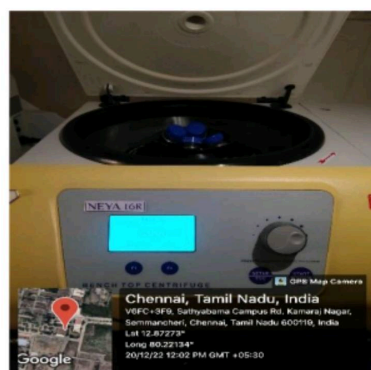


Fig.4.6;a) Centrifugation process

b) Supernatant liquid

4.4.4 Dialysis of supernatant liquid

For three days, the supernatant was dialyzed using cellulose tubular membrane against 1L of distilled water. The supernatant was first placed inside a cellulose tubular membrane, which was then tightly sealed on both ends as shown in figure 4.7. This cellulose tubular membrane was added to a 1L solution of distilled water, with the outer distilled water being changed three times daily. The excess sulphur in the precipitated solution was removed using this procedure.

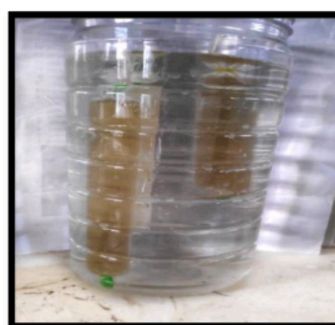


Fig.4.7; a) Liquid in Cellulose tubular membrane b) Dialysis process

4.4.5 Filtration and Freeze drying of keratin sample

The dialyzed sample was filtered using Whatman filter paper to obtain the protein fraction. Prior to use, this protein will be kept at 4°C. The given keratin protein was examined using FTIR as well as the biuret test to confirm its identity.



Fig.4.8; a) Filtration of dialyzed sample

b) Extracted keratin sample

4.5 CHARACTERIZATION OF KERATIN SAMPLE

Using FTIR in the attenuated reflectance mode, the functional group present in keratin protein was identified.

4.6 DETERMINATION OF EXTRACTION YIELD

The initial weight of the discarded human hair and the total dry mass of the keratin sample were used to calculate the obtaining yield. (Yin et al., 2013).

$$\text{Extraction yield (\%)} = W_1/W * 100$$

Where W= Initial mass of the human hair

W_1 = Mass of the freeze dried sample . The weight difference was computed.

4.7 DETERMINATION OF MOISTURE CONTENT OF KERATIN SAMPLE

Before being heated at 105 °C for 3 hours to a constant weight, a 2g sample was weighed precisely in a known weight crucible. The sample was then put in a dehydrator to cool, and the weight was then determined again(Prasetyo et al., 2017b).

$$\% \text{ moisture content} = \frac{W_s - W_1}{W_s} * 100$$

where

W_s - sample weight, (g)

W_1 - sample weight after drying. (g)

4.8 DETERMINATION OF COLOUR,TEXTURE AND WATER SOLUBILITY OF KERATIN

A physical eye examination was used to determine sample colour and texture. 2 g of the sample were boiled at 90°C in 25 mL of hot water to determine their solubility in hot water.

4.9 BIURET TEST

It is also referred to as Piotrowski's test and is a chemical procedure for determining whether peptide bonds are present or not. The presence of keratin protein can be verified using the biuret reaction(Shaik & Author, 2019).

4.9.1 Biuret reagent preparation

Dissolve 100 ml can be made by dissolving 1 g of copper sulphate in distilled water. Stir carefully to combine 100 ml of demineralised water with 10 g of sodium hydroxide pellets.

4.9.2 Protein sample preparation

For the protein solution add 1g of dried keratin powder sample and mix it with 100 ml distilled water.

4.9.3 Procedure for biuret test

Add 2ml of NaOH to 2ml of protein sample then add 3-4 drops of CuSO₄. A purple-violet colour is a positive result.

4.9.4 Observation of colour change

- No colour change → protein not present
- Colour change (turns into deep purple) → protein is present

4.10 PRODUCTION OF BIOPLASTIC FILM

4.10.1 Methodology 1

- Production of bioplastic using gelatin

About 300-450 ml of water were boiled to the boiling point, and then 10–12% of starch by weight were added to make the bioplastic film. The development of plastic film was achieved by blending the ingredients to achieve uniformity and prevent lumping. This process caused the molecules to line up and bind in an orderly manner to create a stronger substance. 9–12% by weight of plasticizers derived from glucose were added to the boiling solution to increase the blending and moulding properties. The highest tensile strength, tensile modulus, and thermal stability are anticipated from the plasticizers. The polysaccharides found in the keratin removed from human hair serve as the foundation for bioplastics. Previously processed 9–12 grammes of the keratin were added to the boiling mixture of starch and plasticizers, and then 10-12% by volume of glycerol was added to trap the water molecules in the starch chains, making the mixture less crystalline and less brittle. The addition of citric acid, which is 40 to 50 percent by volume of glycerol, increased the tensile strength even more. The required addition of 0.5 M NaOH helped to consistently maintain the pH of the bioplastic formulation. A 1:1 equal molar (0.5M) mixture of KOH and CH₃COOH was added to the bio-plastic to increase its resistance to both rodent and fungal attack following several trial formulations that demonstrated poor resistance to these attacks despite having the highest tensile strength available from the bioplastic. The right amount of walnut shell powder (3-5gms) and 30% by volume of 0.5M HCl were added to the bioplastic to strengthen it even more and improve uniform bonding.

5 to 8 ml of acetic acid source were added to increase solubility, and 20 to 23 percent by weight of gelatin was added to increase bioplasticity. All these components were added and mixed in regular manner in a boiling temperature of water as depicted in figure 4.9 to get a solution for the production of bioplastic.



Figure 4.9 Solution of keratin mixture

➤ Drying and finishing process of bioplastic film

The mixture containing all the ingredients is mixed well and uniformly using a spatula. Continuous mixing is done to avoid any kind of lump formation and uniformity in mixture. Sieving of the mixture is done to remove all the foam and bubbles in the mixture. Then the mixture is transferred into a flat bottom tray. The tray is placed at a temperature of 20°C in a dark place to allow drying. The tray is left undisturbed for 60 hours. Completely dried bioplastic layer is slowly separated from the tray and used for various applications such as making of gaskets, insulation of wires, bioslippers etc..

➤ Trial runs

Table 4.3: No of Trial runs and materials used in Methodology 1

No of trials →			
Amount of materials used in trials ↓	Trial 1	Trial 2	Trial 3
Demineralized water(ml)	100	100	100
Starch soluble (g)	15	20	15
D-Sorbitol(g)	15	20	15
Keratin (g)	7	10	12
Glycerol (ml)	4.8	12	6
Citric acid(g)	4	3	2
Walnutshell powder(g)	3	3	3
0.5 M NaOH(ml)	2	5	2
0.5 M HCl(ml)	2	5	2
Gelatin (g)	10	10	7

4.10.2 Methodology 2

➤ Hydrolysis of keratin powder

To make the bio-plastic film, 15g 100 cc of water are dissolved with of PVA. and placed in a magnetic stirrer at 80°C for one hour. The 0.5g of keratin sample is mixed with 60 ml of 0.2 M NaOH Solution, followed by 10 ml of glycerol and 30 ml of PVA. The mixture is then continuously stirred in a magnetic stirrer set at 60 °C for two hour as shown in figure 4.10.

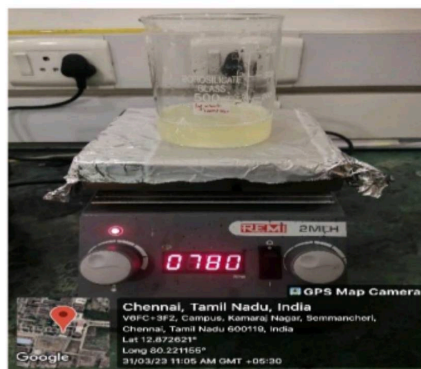
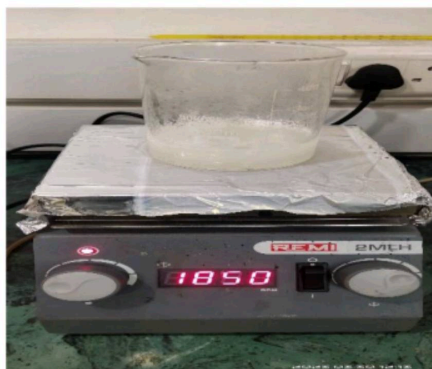


Fig.4.10; a) Polyvinyl alcohol solution

b)Hydrolysis of keratin solution

- Drying of bioplastic film

A 19*10 mm diameter petriplate was filled with the prepared solution, and the plate was then placed in a oven for 24 hours. The film were stripped out from the petriplate and stored for further examination.

- Trial runs

Table 4.4:No of Trial runs and the materials used in Methodology 2

No of trials 	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Materials used 									
PVA(g)	15	15	15	15	15	15	15	15	15
Demineralized water(ml)	100	100	100	100	100	100	100	100	100
PVA(ml)	30	30	30	30	30	30	30	30	30
Glycerol(ml)	10	6	15	9	15	10	10	10	10
Benzoic acid(g)	-	-	-	-	0.5	0.5	-	0.5	0.5
NaOH solution(ml)	60	60	60	60	60	60	60	60	60
Keratin powder(g)	0.03	0.04	0.05	0.5	0.1	0.5	0.3	0.5	0.5
Time taken(hr)	2	2	2	2	1:30	2	1:30	3	3
Remarks of product						Good	Good		

4.10.3 Methodology 3

➤ Preparation of bioplastic film

The extracted Keratin weighed 1g should be taken separately in a beaker. Add 1 g of polyethylene glycol and 1 ml of glycerol. Glycerol is a plasticizer. 1 g of carboxy methyl cellulose should be added. This mixture is put into a beaker with 100 ml of water in it. The mixture is heated under 80°C at 100 rpm in a magnetic stirrer for a minimum of two hours. The obtained sample is then put in a dryer, (ideally one that also contains

magnesium nitrate hexahydrate), and the product is obtained at the desired consistency. The sample is sent for processing to injection moulding.

➤ Trial runs

Table 4.5 No. of trial runs and the materials used in Methodology 3

No of trials →	Trial 1	Trial 2
Materials used ↓		
PVA	1	1
Glycerol	5	10
Polyethylene glycol	1	1
Keratin sample	1	2
Carboxy methyl cellulose	1	1
Distilled water	100	100
Time taken	1:30	1

CHAPTER – 5**RESULTS AND DISCUSSION****5.1 DELIPDIZATION OF HUMAN HAIR**

After delipidization the surface oil and internal lipids from the hair was removed. The removal of oil ensures that the extracted keratin will be of high quality and purity. The weight before the delipidization process and after the process also varies and it makes sure that removal of oil takes place in this process.

5.2 DISSOLUTION OF HUMAN HAIR

In this process the sodium sulfide makes the human hair to dissolve completely. The yield of keratin is more in 500 mM concentration because the hair got completely dissolved in sodium sulfide when compared to other concentration. The different volume of sodium sulfide taken in this process gives the yield in increasing order which makes us to understand the importance of sodium sulfide usage.

5.3 DIALYSIS OF SUPERNATANT LIQUID

The sulphur got removed from the solution when it get dialyzed against distilled water as a buffer solution. The removal of sulphur in keratin extraction helps to prevent the reduction of mechanical properties, color changes etc.. By dialyzing, the small impurities and unwanted molecules also removed from the keratin and make it pure and suitable for industrial and biomedical application.

Table 5.1: Components of Keratin

Keratin components	Composition
Amino acids	20%
Peptides	15%
Minerals	5%
Disulfide bonds	10%

5.4 EXTRACTED KERATIN SAMPLE



Fig.5.1; Sample of keratin powder

5.5 YIELD % OF EXTRACTED KERATIN

The yield of keratin based dissolution of human hair in sodium sulfide solution. It shows maximum yield at highest concentration of 500 Mm.

Table 5.2: Calculated yield percentage

Serial no	Concentration of sodium sulfide used(mM)	Initial weight of human hair taken	Amount of extracted keratin powder(g)	Yield %
1	100	25	16.3	65.2
2	200	25	19.2	76.8
3	300	25	22.4	89.6

5.5.1 Effect of concentration and time on yield % graph

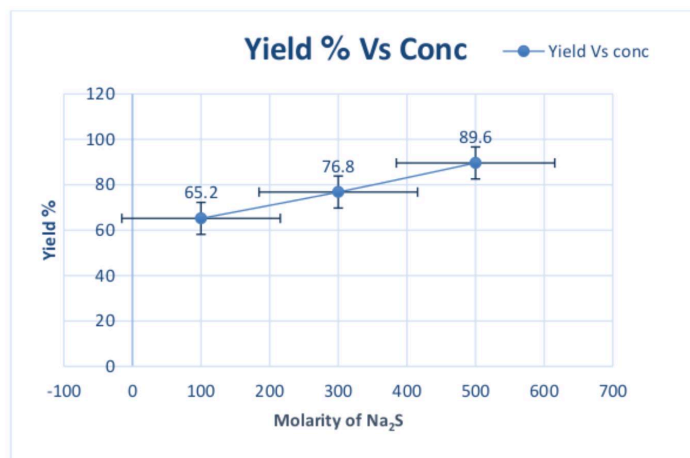


Fig.5.2; Yield % Vs Concentration graph

The human hair showed maximum solubility at 6 hr. After 6 hr there is no change in the given solution. In this figure, the graph showed the increasing order of keratin yield with respect to the increase in time and it remains constant after 6 hr.

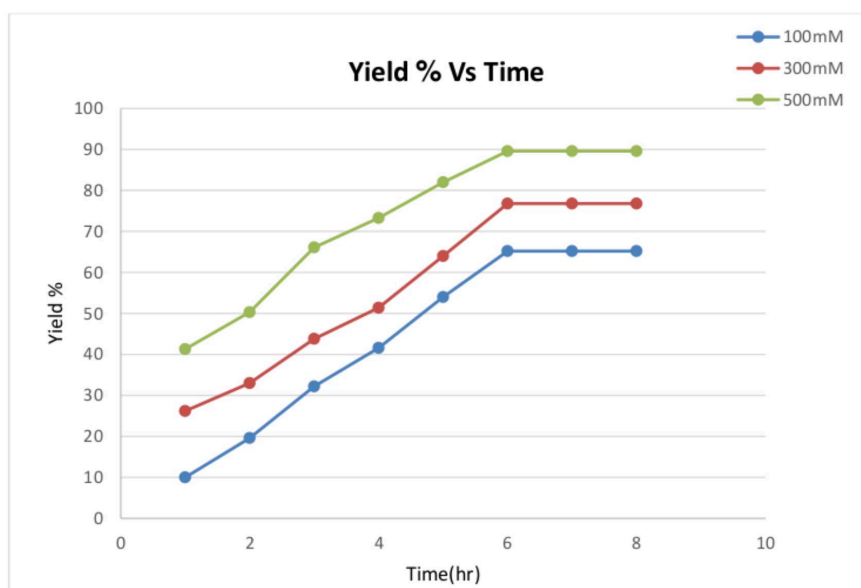


Fig.5.3; Yield % Vs Time graph

5.6 SOLUBILIZATION OF HUMAN HAIR

Chops of human hair were dissolved in Na_2S at 0.1, 0.3, and 0.5M concentrations. With 0.5M Na_2S , the human hair fractions were most soluble. The human hairs were partially dissolved at lower concentrations of 0.1 and 0.3M. The keratin from 25g of dry human hair yielded 65.2%, 76.8%, and 89.6% at 0.1, 0.3, and 0.5 M. The production was of keratin increased, with higher Na_2S concentrations. The human hair fractions' solubility peaked at 6 hours. The given solution has not changed after 6 hours.

5.7 KERATIN CONFIRMATION TEST

5.7.1 Confirmation of protein using Biuret test

The given solution changed to deep violet purple in the given figure 5.4. It demonstrates that keratin protein exist.

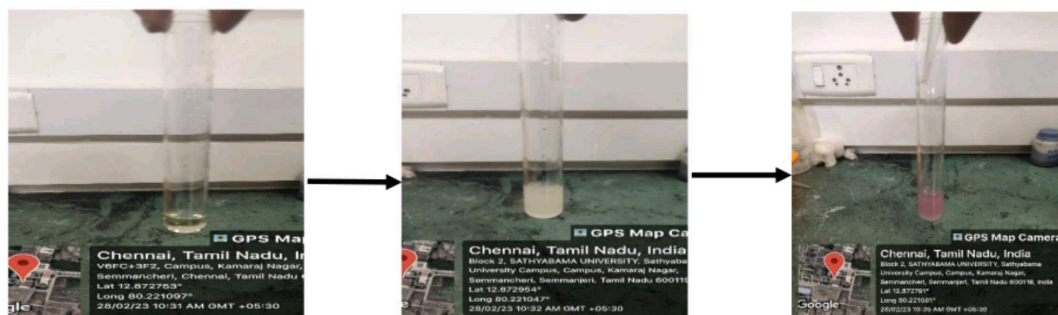


Fig.5.4; Changes in the sample after using biuret reagent

5.7.2 Characterization of keratin sample

It provided the useful details regarding molecular structure and structure of protein. The intensity and shape of these bands provide the information on the secondary structure and alterations in conformation in the keratin sample.

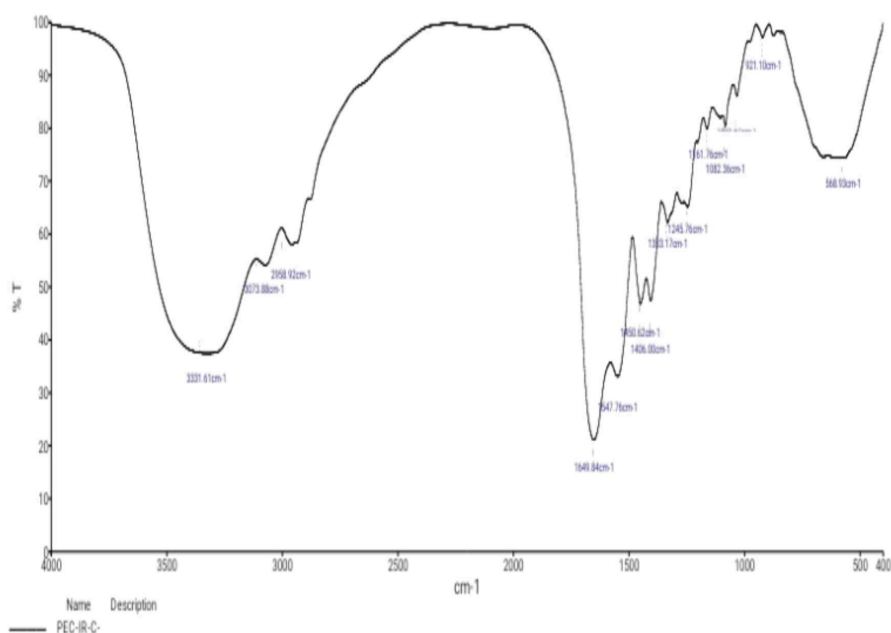


Fig.5.5; FTIR spectrum of produced keratin powder

Figure 5.5 displays the FT-IR spectrum of the keratin that was generated. The findings revealed the Amide A, 1, 2, and 3 groupings. Amide A is the name of a nitrogen hydrogen bond that demonstrated the existence of a group made up of amide and had a broad and weak mono absorbance zone at 3331.6 cm^{-1} . Amidates I through III investigated the protein structural configurations. The spectra of the samples revealed the absorption bands of C=O at 1649.84 cm^{-1} (amide 1), the nitrogen hydrogen bending at 1547.76 cm^{-1} (amide 2), and C-N bonds and C-O bending at 1245.76 cm^{-1} (amide 3).

Table 5.3:Major functional groups identified in keratin

Frequency ranges (cm^{-1})	Observed range (cm^{-1})	Functional groups
3400-3300	3331.6	N-H Stretching vibration Presence of amide A
3300-2500	3331.61	O-H Stretching vibration presence of

		carboxylic acid
3200-2700	3073.88	¹⁷ O-H stretching vibration presence of alcohol and phenol
3000-2840	2958.92	¹⁷ C-H Stretching vibration Presence of alkane
1630-1710	1649.84	C=O Stretching vibration Presence of amide-I
1550-1650	1547.76	N-H blending vibration Presence of amide-II (amine primary)
1415-1380	1406.00	S=O Stretching vibration Presence of sulfate
1350-1300	1333.17	S=O Stretching vibration Presence of sulfone
1250-1020	1245.76	²⁸ C-N & C-O Stretching vibration Presence of amide-III
1205-1124	1161.76	²⁸ C-O Stretching vibration Presence of tertiary alcohol

⁴⁷ 5.8 DETERMINATION OF MOISTURE CONTENT OF EXTRACTED KERATIN:

The moisture content of the keratin sample is calculated to be 13.5%.

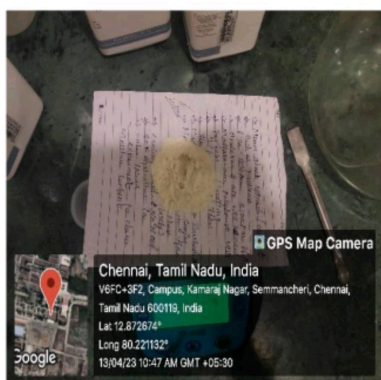




Fig.5.6; Analysis of moisture content

5.9 COLOUR,TEXTURE AND WATER SOLUBILITY

Using hand feel and physical eye examination , it was observed that the keratin was finely powdered material and yellowish brown in colour.keratin is completely insoluble in cold and hot water as shown in figure 5.7.

Table 5.4:Colour, Texture and Solubility

Texture	Fine powdered
Colour	Yellowish brown
Water solubility	Insoluble
Moisture content	13.5%

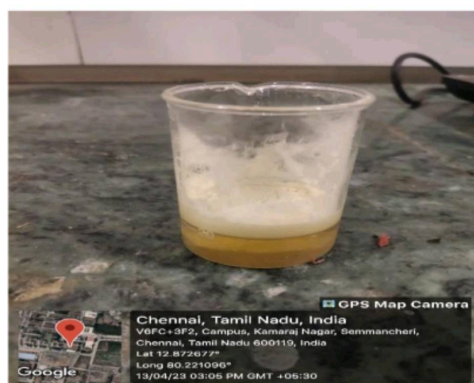


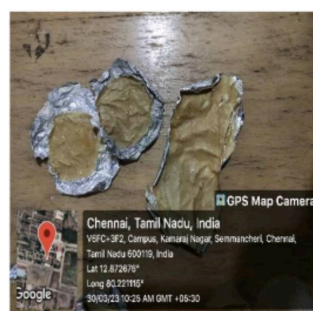
Fig.5.7; Solubility of keratin powder in water

5.10 BIOPLASTIC FILM TRIALS

5.10.1 Trial run pictures of Methodology 1



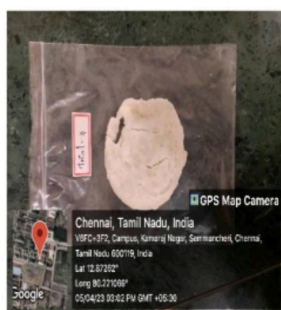
Trial 2



Trial 3

Fig.5.8; Products obtained from Methodology 1

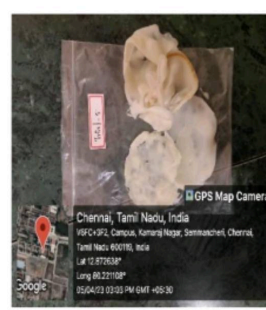
5.10.2 Trial run pictures of Methodology 2



Trial -3



Trial- 4



Trial- 5



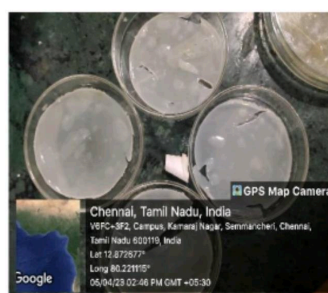
Trial - 6



Trial - 7



Trial - 8



Trial - 9

Fig.5.9; Products obtained from Methodology 2

5.10.3 Trial run pictures of Methodology 3



Trial 2

Fig.5.10; Products obtained from Methodology 3

5.11 FINAL OPTIMIZED METHOD FOR BIOPLASTIC FILM PRODUCTION

After trying three methods for the production of bioplastic film, the finally optimized method is the method 2 i.e hydrolysis of keratin powder. In this process keratin breaks down to soluble form and it helps in the production of bioplastic film. On the first 2 trial film is produced it got stick into the beaker. In trial 3, 4 and 5, the bioplastic film is produced but it got affected by fungal activity. To prevent the microbial attack, antibacterial agent i.e benzoic acid, is added. In trial 6, the film produced in this concentration was smooth and has a good colour, strength and mechanical properties like tensile strength, hardness etc., The first and second concentration keratin powder were used in the trial 8 and 9 and it does not come out well. It has less elasticity when compared to the third concentration keratin powder. The film produced in the method 3 was sticky and it has more moisture content even it is dried in the oven for 2 long days. This moisture leads to a fungal attack easily. The film produced in method 1 also not good when compared to method 1.

5.11.1 Optimized bioplastic film



Figure 5.11 Bioplastic film

5.12 CHARACTERIZATION OF BIOPLASTIC FILM

5.12.1 Scanning electron microscope

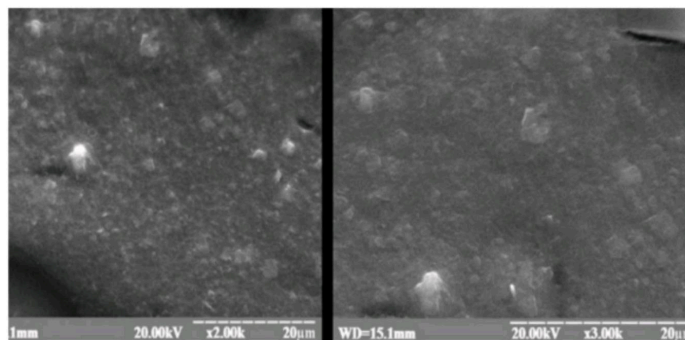


Fig.5.12; SEM Image of Optimized film

Figure 5.12 displays the findings of a SEM examination of keratin based bioplastic film. It has extremely good suitable morphologies that are free of cavities, edges, and holes. The occurrence of chemical interactions between keratin and glycerol was therefore attributed to the components' strong bonding. As a result, the glycerol contributed to the creation of a homogeneous mixture with visible plasticization in the keratin matrix. According to SEM analysis of the specimen's structure, the bioplastic's lightness at its smooth surface implies the presence of bonded structures. Their smooth surface morphology with fissures makes this clear, demonstrating their robust character

5.12.2 X-Ray Diffraction

Using an XRD system with copper K-ray radiation and generator parameters of 45 kV and 30 mA, the crystal structure of Keratin-based bioplastic films was assessed. The data for the dispersion angle differ from 5 to 50 were collected at a rate of 0.02/min. In accordance with Bragg's equation, the essential spacing was determined by the location of the peak in the XRD diagram:

$$n\lambda = 2d\sin\theta$$

Where: n is the integer, d is the distance between the planes of crystals, θ is the dispersion angle, and λ is the electrons wavelength(Humphreys, 2013).

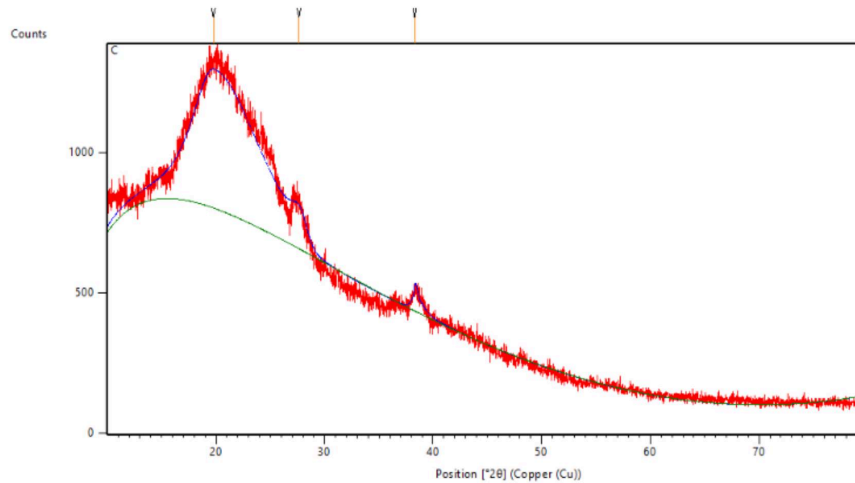


Figure 5.13 XRD graph of optimized graph

Figure 5.13 displays the XRD images of the keratin-based bioplastic film. It displayed primary peaks at $2\theta = 19.7$, 27.6 , and 38.3 showed the powerful crystalline characteristics. The following interlayer spacing decreased as the angle shifted upward, indicating an ordered structure for the blended component. The rise in d-spacing showed that the mixture's structure is less organised., which increases the difficulty of crystallisation. The term "d-spacing" refers to the distance between atoms .Bragg's equation states that as d-spacing is reduced, the value of 2 increases(Pope, 1997). The bioplastic film with the highest 2θ values and lowest d-spacing, as shown in Figure 4.13, also has the most ordered structure.

Table 5.5:X-ray diffractogram of synthesized Bioplastic film

Pos. [2θ]	Height [cts]	FWHM Left [2θ]	d-spacing [Å]	Rel. Int. [%]
19.7643	331.37	4.7345	4.48835	100.00
27.6219	112.22	3.0558	3.22680	33.87
38.3088	83.21	0.3591	2.34765	25.11

5.12.3 Fourier transform infrared spectroscopy

The figure 5.14 shows the FT-TR spectrum of keratin based bioplastic film. The findings revealed the Amide A, 1, 2, and 3 together. The occurrence of an amide unit was indicate by a broad and weak single absorption band for the N-H bond at 3437.36 cm^{-1} , which was named Amide A. Amidés I–III examined the protein

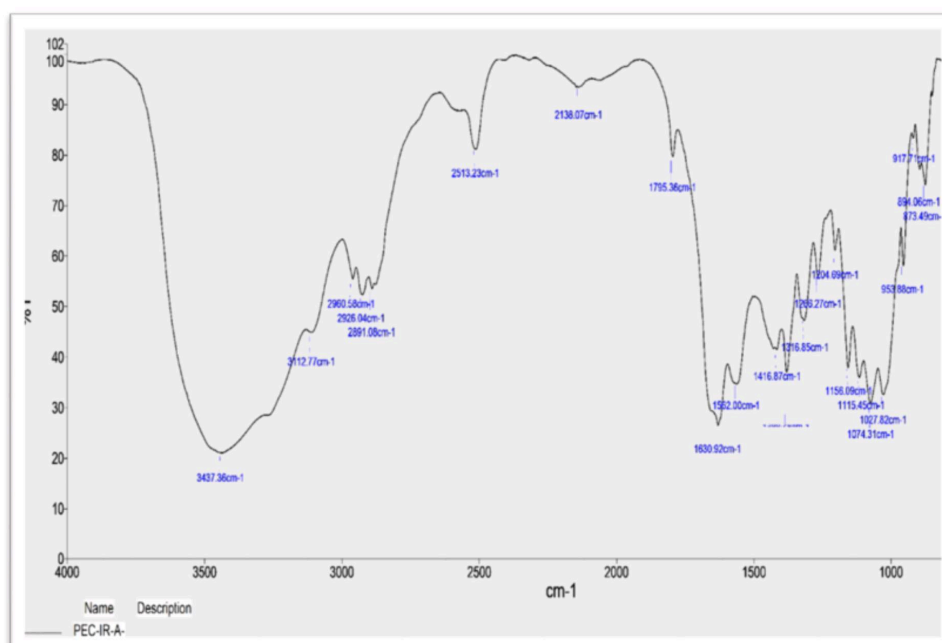


Fig.5.14;FTIR image of optimized film

conformational arrangements. The absorption bands of C=O at 1630.92 cm^{-1} (amide I), N-H bending at 1562.00 cm^{-1} (Amide II), and C-N and C-O stretching at 1204.69, 1027.82 and 1074.82 cm^{-1} (amide III) were showed in the wavelength of the sample. From the table 5.6, it shows the presence of functional groups like amine, amides, sulfone, alcohols etc. All of these groups indicates the presence of keratin and glycerol from the given bioplastic film

Table 5.6:Major functional groups identified in bioplastic film

Frequency ranges(cm^{-1})	Observed ranges(cm^{-1})	Functional groups
3400-3500	3437.36	Primary amine (amide A) present in the N-H stretching vibration
3300-2500	3112.77	O-H Stretching vibration presence of carboxylic acid
3200-2700	3112.77	O-H stretching vibration presence of alcohol and phenol
3000-2840	2960.58, 2891.08	C-H Stretching vibration Presence of alkane
1630-1710	1630.92	C=O Stretching vibration Presence of amide-I
1550-1650	1562.00	N-H blending vibration Presence of amide-II (amine primary)
1420-1330	1416.87	O-H blending vibration Presence of alcohol
1350-1300	1316.85	S=O Stretching vibration Presence of sulfone
1250-1020	1204.69, 1027.82, 1074.82	C-N & C-O Stretching vibration Presence of amide-III
1205-1124	1156.09, 1115.09	C-O Stretching vibration

		Presence of tertiary alcohol
900-990	953.88, 917.71	C=O blending vibration Presence of alkene

5.13 BIODEGRADABILITY TEST

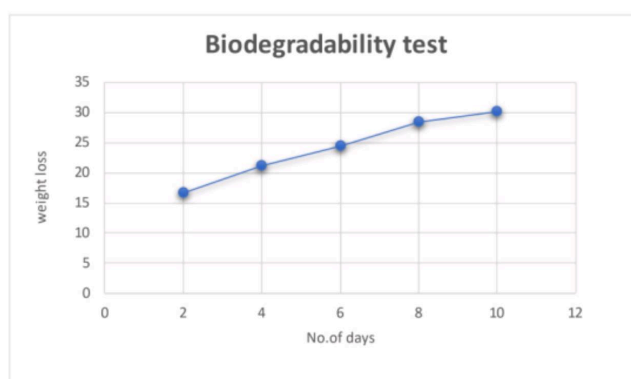


Fig.5.15; Biodegradability graph

Figures 5.15 and 5.16 display the outcomes of the biodegradability test conducted using the Soil Burial Test method. As can be seen, the reduction in weight of the bioplastic increases with the length of the burial period, which indicates the greater the degree of degradation. Every day, more weight was lost, reaching a peak of 30.2% on day 10. In linear polymers, chemical degradation reactions lead to a reduction in molecular mass or length chain minimizing. The size of this reduction in mass was caused by the composition of bioplastic, which was made of an organic substance that microbes could quickly digest (Harsojuwono et al., 2019).





Fig.5.16; Soil burial Test. (1) Bioplastic on the day zero (2) size of bioplastic film was expanded on day two (3) More water and soil are entered into the bioplastic film on day four (4) Wide crack identified on bioplastic on day six (5) Some parts of bioplastic were diminished on day eight (6) most of the parts of bioplastics were completely degraded on day ten.

As depicted in Figure 5.16, the bioplastic sample started to physically change on day 4 where cracks were seen on the upper layer of the sample. The sample's form then started to break down on the eighth and tenth days. In contrast to another study [14], the sample's It shrank in size and became brittle and hard, after being buried for 90 days. Changes in macrostructure were significantly influenced by soil degradation. It's possible that the soil had more nutrients and that several microbes (fungi and bacteria) worked together in concert to damage the macrostructure of the bioplastic samples.

Table 5.7:Calculation of weight loss % of bioplastic film

Number of days	Weight loss %
Day 2	16.67
Day 4	21.1
Day 6	24.5
Day 8	28.4
Day 10	30.2

5.14 ANTIMICROBIAL ACTIVITY OF THE BIOFILM

In this study, the provided bioplastic film had antibacterial agents like benzoic acid added to it. An analysis of the biofilms on total bacterial count was done by the Tamilnadu Agricultural University. Bioplastic films without antibacterial agents (film₁) and bioplastic films with antibacterial agents (film₂) are the two types of biofilms that are analysed. According to the results, the total number of bacteria on films 1 and 2 is 7.2102 cfu/g and 2.36102 cfu/g, respectively. This finding demonstrates how antibacterial substances like benzoic acid can help to lessen microbial attack on biofilms.

Table 5.8: Total Bacterial count of bioplastic film

Types of bioplastic	Number of bacterial count
Film ₁	7.27×10 ² cfu/g
Film ₂	2.36×10 ² cfu/g

5.15 PROPERTIES OF BIOPLASTICS

5.15.1 Thickness of film

There were three different biofilm samples that were examined. With a Vernier calliper and fifteen unique measurement value on the film, the average film thickness was determined to the nearest 0.0001 mm. The outcome showed that the thickness of the films, which were recorded at 0.018, 0.019, and 0.024 mm, did not significantly differ from one another. Different amounts of keratin solution could be the cause of the variations in film thickness.

5.15.2 Density of film

There are three different pieces of biofilms was analyzed. The density of biofilm having recorded 1.39g/cm³, 1.43g/cm³, 1.51g/cm³. The density of film depends up on the thickness of film and area of biofilm. These outcomes imply that keratin would increase the biofilm's flexible and breaking strength. The molecular weight and density of the keratin, PVA, and plasticizers included in the biofilm may be responsible for the change in density.

5.15.3 Moisture content of film

Bioplastic with a higher concentration of keratin showed the highest moisture content of 22.78 ± 0.69 . As a result, bioplastic film with a higher moisture content can be used to pack food. These outcomes concur with those mentioned by (Prasetyo et al., 2017a)

5.15.4 Solubility of film

With a greater ratio of keratin content and amount of plasticizer compared to other bioplastics, the third demonstrated a poorer solubility in water. Normally, the presence of PVA would make the films more water-soluble, but in this case, the PVA was constant while the amounts of keratin and glycerol varied. The third concentration product with the greatest amount of keratin showed lowest solubility.

Table 5.9:Percentage of water solubility

Sample	Initial sample (g)	Final weight (g)	Solubility in water (%)
S ₁	0.302	0.264	12.52
S ₂	0.291	0.251	11.61
S ₃	0.312	0.276	11.53

5.15.5 Tensile strength of biofilm

The film's strength in tensile test shows the highest stress created during tensile testing (Sachdeva et al., 2017). The bioplastic made from first two concentration keratin sample and the amount of plasticizer used showed the minimum tensile strength of 7.67 ± 0.18 Mpa and 9 ± 0.20 . The maximum tensile strength of 10.4 ± 0.22 MPa was obtained on the third concentration film.

5.15.6 Young's modulus of elasticity

By measuring the Young's modulus, an item's or film's stiffness can be determined; the higher the value, the stiffer the film (Gonçalves et al., 2019). The maximum Young's modulus of 169 ± 0.88 MPa was obtained of the bioplastic film made from third concentration bioplastic film which exceeded the films created using the other two concentrations.

CHAPTER 6

SUMMARY AND CONCLUSION

Despite being merely composed of proteins, keratin is among the toughest materials found in nature and serves as an effective protective covering. It presents a huge opportunity for novel bioinspired designs with a deep comprehension of natural science, structure, and mechanism. The present chemical methods for getting keratin are efficient yet costly, making them less applicable for commercial uses. Therefore, there is a chance to create brand-new strategies for the efficient segregation of keratin. This experiment successfully created bioplastic films using human hair. The physicochemical and mechanical qualities of the films are significantly enhanced by the application of a moulding agent. The biodegradable film had the highest density, moisture content, transparency, and solubility in water of all the films. Their bending strength, and Young's modulus of elasticity are all increased by the presence of keratin. The presence of amides content was verified by FTIR, which also validated the chemical linkage and connection between the plasticizer and the matrix of polymers. The relative crystallinity of the bioplastic film is the greatest at 33.87%. The biological decomposition analysis shows that the bioplastic sheets degrade in a variety of situations, including composting, natural environments, and lab settings. A weight loss of 32.2% was achieved in lab settings after 10 days. As a result, the qualities that the films showed after the proper amount of plasticizer was added suggest that sustainable bioplastic production is possible. The film is ideal for packaging food due to its remarkable qualities, including low transparency, humidity, and solubility in water. Due to its excellent tensile strength and elastic modulus, it can also be utilised to produce rigid plastic containers and toys. The creation of valuable things can now employ other sources thanks to this research. In conclusion, keratin has been effectively extracted from human hair to create an artificial, organic-based bioplastic film. Even though the experiment showed remarkable bioplastic capabilities, the expected development was seen when keratin was included. Furthermore, the high crystal structure, good surface form, and thermal properties were validated by SEM and XRD analysis. Finally, there are numerous industrial uses for the developed keratin-based bioplastic film.

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