A Review on Bioplastics: The use, reuse and degradation of the right plastics

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Abstract:
Bio plastics, a class of polymers derived from renewable sources, have gained significant attention in recent years due to their potential to address environmental concerns associated with traditional petroleum-based plastics. This review explores the various aspects of bioplastics, focusing on their use, reuse, and degradation pathways. From their production to end-of-life scenarios, we delve into the current state of knowledge regarding bioplastics' benefits, challenges, and sustainability implications. Bioplastics (right plastic) are bio-based like PLA, PHA & PBS and biodegradable like PHB, PBAT…having tremendous utilization quality in packing, agriculture and horticulture. As for reuse and recycling of bioplastic, done in two ways: mechanical recycling and chemical recycling. Mechanical recycling is not yet available for commercial use but reducing the molecular weight and tensile strength recycling of biopolymer like PLA and PHA is possible. In contrast to mechanical recycling, chemical recycling offers the potential for making high-quality polymers from waste. In this review, we firstly discuss about the sources of bioplastics and its types along with utilization factors. How it will reuse and recycle and key points that affect its biodegradation rate. And finally environment implication with future prospects.

Keywords: bio-based plastics, bio-degradable, sustainable material,bio-polymer

1. Introduction:
The development of plastic has become a part of human life because of its properties like lightweight, low-cost, long – durability, etc. Most of the plastics are petroleum-based, having a production rate of 367 million tons annually as the production rate is very cheap. The reason behind the wide use of plastics is corrosion resistance, strength, poor conductance to electricity, very convenient to mold into the desired shape, etc. [1], [2]
There is no doubt that chemical-derived materials had negative effects on our environment, the excessive use of petroleum-based plastics has become the enemy of the environment and humankind. Plastic is non-biodegradable in nature it remains for more than 100 years in the soil.

According to researchers' reports, the ocean has up to 5 trillion pieces of plastic which badly disturbs aquatic life.[3], [4] Records show that approximation of the manufacturing of plastics may be 390.7 million tons which is increasing up to 4 % every year and only 7% of plastics are recycled. In the food industries, 40% of plastic is used for packing from the total plastic consumption annually. There is no doubt that they are helpful for the economic growth of the world, but in return, it is destroying the entire human race and polluting our environment.[3], [5]

During the combustion of the plastics, it produces benzo(a)pyrene (BAP), polyaromatic hydrocarbons (PAHs), and hydrogen cyanide which are harmful gases that lead to diseases like cancer, asthma, and emphysema.

To treat plastic waste scientists are conducted much research to find the right plastics to use. And that right plastic is bioplastic. [6], [7]

BIOPLASTIC

This paper aims to make the world free from plastic by replacing petroleum-based plastic with bioplastic. Bioplastics are made up of natural ingredients which are present abundantly on our earth, like algae, crops, vegetables, fruits, seeds, etc. It is also manufactured by waste material like vegetable peel, fruit husk, sea waste material, etc. From a global point of view, the composition of bioplastic from waste material is the best, most sustainable, and most effective solution in the "GREEN EARTH "revolution.[2], [4] Using natural materials in the synthesis of bioplastics will not cause any toxic effects on the environment. The level of degradation of bio-based plastic in nature is very high as compared to other green plastic. The main point of the popularity of the right plastic is to minification of greenhouse gasses during the manufacturing process. They are highly biocompatible, and the degrees of these parameters can be determined by different types of analysis for example strength analysis, ductility analysis, and degradation level analysis. Different types of bioplastics are formed which may be based on starch, protein, microbes, cellulose, etc. they have to differ in characterization, properties, factors, etc. [3], [7]

Motivation and The Selection of The Topic

Nowadays, plastic waste become one of the major pollutions on earth. It is impossible to vanish all the plastic in the world, but it can replace with the right plastic. And bio-based plastic is a promising plastic that is degradable in nature with no toxic effects.

One of the major advantages of using the right plastic is its materials. Bioplastic is not just a single component, it is composed using different natural materials or bio-based waste products. Glycerol is the waste product of the biodiesel industries and is used to produce biopolymers for ecological and economical significance.

Using waste material to compose biopolymer is the best way to treat waste or utilize the energy for a greener world. Bioplastic decomposes rapidly which gained a specific attraction.

TYPES AND SOURCES OF BIOPLASTICS

1.1 Bio-based plastics

Polylactic acid (PLA)

Polylactic acid bio-based plastics mostly used today as they have number of eco-friendly aspects. PLA formed by the condensation process of lactic acid. This bioplastic is easily degraded in the nature unlike petroleum-based plastic by the action of microorganisms.[9],[10] PLA basically synthesized by the action of bacterial fermentation of carbohydrates and polymerization. It includes multi-steps, starts with fermentation, and ends with polymerization process.[11] PLA used for many applications as it is highly resistant to oil-based goods. Nano clay has been added in PLA to increase its oxygen barrier capacity as it is lacking in oxygen barrier capacity.[12] PLA plastic having low degradation level and stereoisomeric properties it can be best alternative option of the petroleum-based plastic. A pure kind of PLA will slowly degrade in the earth as few months to one or two years as compared with the petroleum-based plastics which will take up to 1000 years to degrade even though that much long time it will not completely degrade in the soil.

Starch-based plastics

Starch-based bioplastics is the best bio-based and biodegradable plastic which is natural polymer present abundantly on the earth at a very cheap cost.[13] Whenever a suitable plasticizer is added in starch-based bioplastics, it will attain magnificent mechanical properties which help to decrease the rate of degradation process. The production process of starch-based plastic is very simple, easy and at low cost, so that the necessary bioplastic characteristics can be easily obtained of it. As having the eco-friendly property, the 50% production rate of the bioplastic in the world are starch-based bioplastics. Starch doesn't have any taste or smell although it is in white in color, but it possesses two types of molecule amylose and amylopectin.[14] It helps to make network which compresses water and increase viscosity, the percentage of amylose and amylopectin present in various types of starch are shown in table no.1[15]

Source	Amylose (in %)	Amylopectin (in %)
Wheat	19-25	74-80
Potato	17-23	75-80
Corn	23-28	70-75
Chestnut	17-20	77-81
Rice	16-19	80-83
Grain sorghum	20-25	75-80
Banana	17-24	76-83

Polyhydroxy alkanoates (PHA)

Polyhydroxy alkenoate's based bioplastics make their own shine in the field of bio-based plastics. According to the statics in 2019, the total production of the PHA was more than 25k tones and it will increase up to 2 lakhs tones in 2024.

The reason behind its increasing in production is its unique characteristics. PHA having number of properties like high degradation level, highly biocompatibility, an optically active compound, having safe nature and renewable waste sources.[16],[17] PHA belongs to the polyhydroxy ester family of 3-,4-,5-and 6- hydroxy alkanoic acids.[18] PHA having favourable physical and mechanical properties that it will easily made up by specific bacteria like pseudomonas, ralstania eutropha, rhodobacter sphaeroides, thermos thermophiles etc.[19] They can also be synthesized by cells with the help of approprete organic solvents like halogenated pure solvents. Definitely it is less expensive way to produce biodegradable and biocompatible bio-based plastics.[20]

Poly butylene succinate (PBS)

Poly butylene succinate is an aliphatic polymer which is a best bioplastic, biodegradable and non-toxic.[21] The production rate of PBS is higher than the PLA on the bases of production and cost.[22] Generally, PBS is made-up of two type of monomers, succinic acid and 1,4-butanediol. As we seek the natural production method; succinic acid can be made-up from fermentation and 1,4-butanediol possibly obtained by genetically modified organism.[23] The best thing about the PBS bioplastics is that properties can be modify according to our need which mean we can upgrade its resistance tendency, thermal capacity, mechanisms and physicochemical properties in order to change its degradation time. By doing this, it can be applicable into various field where it shows its marvelous nature toward the environment.

1.2 BIODEGRABLE PLASTICS

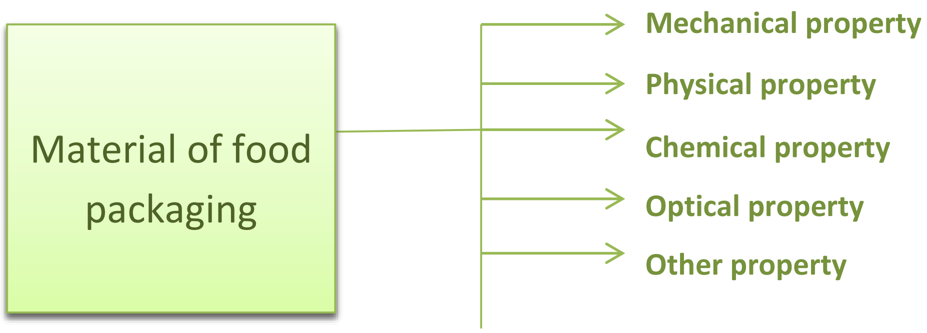
Polyhydroxybutyrate (PHB): In 1966, PHB was discovered by lemoigne from paris by the chloroform extraction, lemoigne isolated PHB from bacillus megaterium.[24] PHB is the biodegradable bioplastic as it is having nature safe behavior which become best alternative for synthetic polymer. As polyhydroxybutyrate (PHB) characterized from PHA, it exhibits number of properties which is eco-friendly . The main advantages of PHB is that it show good barrier feature as it compared with polyethylene terephthalate (PET) and polyvinylchloride (PVC) .As long with that PHB having linear chain like structure with amorphous and crystalline phases . Due to the present methyl and ester group as the functional group, there may be involvement of the two types of temperature for the two different phases. For amorphous phase, there is a glass transition temperature (Tm) and for crystalline phase, there is a melting temperature (Tc). The presence of the functional group directly affected thermal, crystallinity, tensile strength, hydrophobic properties of the material.[25] PHB can be synthesized by the bacteria through fermentation and having melting point temperature (170˚C-180˚C). As having unique physiological function material, PHB is much similar to the petroleum-based polymer for the sustainable target.[26] Today many researchers found the best and cheap way to produce PHB from food waste which making it superior to the other polymers.

Poly(butylene adipate-co-terephthalate)(PBAT): PBAT polymer mostly used biodegradable polymer now a days due to it mechanical and thermal properties . PBAT having amorphous structure with low crystalline or high flexibility.[27] Presence of butylene adipate group can affect its biodegradability. According to analysis, in 2021, the production of PBAT was upto 19-20% from the total production of the bioplastic and this production rate is increasing. PBAT can best alternative to low-density polyethylene (LDPE) even with the limitations like low stiffness. Low modulus, high cost etc. The main application about PBAT is plastic films. PBAT can be obtained by the poly-condensation process with the combinations of adipic acid, 1,4-butanediol and terephthalic acid.[28] PBAT basically fossil- based polymer and an aliphatic –aromatic co-polyester. With the aromatic unit in the chain, the degradation speed of PBAT will become fast in the nature with low resistance and high ductibility .The main point to be noted about PBAT is that its mechanical properties , we can change it according to need with increasing the terephthalate portion.

Utilization of bioplastics:

2.1 Packing applications

Food packing: The selection of the material of packing of food is very important in the field of food development and technology as it preserve different type of food. Because of the use of petroleum-based material as food packaging leads to harmful effects,[29] industries start to show interest in bioplastic as a food packaging material from the last 15-10 years. Bioplastic is bio-based as well as biodegradable which degrade by the action of micro-organisms and having eco-friendly nature towards the environment. The properties of the material are shown in below diagram

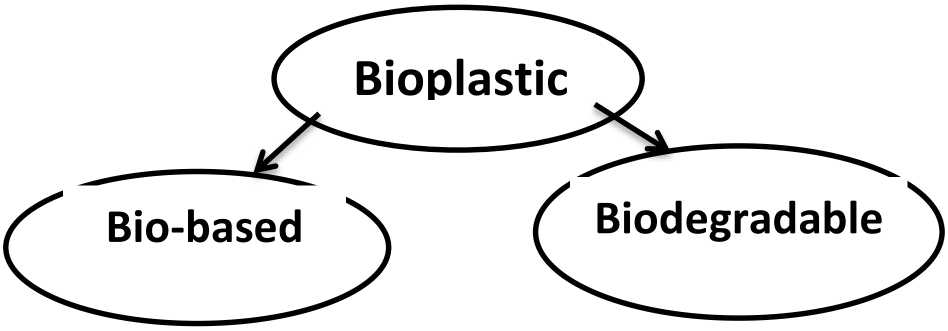


In food industry, the main application of bioplastic is packaging for short and long shelf life products. Different type of bioplastic is used like starch-based and PLA.[29] These two plastic materials having greater potential towards the food packaging application. It protect and preserve different types of organic fruits and vegetables, baking goods, different types of herbs, potato chips etc. Having barrier availability, desirable mechanical properties, strength, temperature, stability, non-toxic, light weight, these can make a better option for suitable packaging purpose. Replacing petroleum-based material with bioplastic material for packaging has been a right thing for environment and humankind.

Single-use items: As the name indicate, single use items are one-time items. These can be used only for one time and after that it will be recycled. Plastics become necessary thing but use of petroleum-based plastic having very bad effects especially for marine life. It cannot be totally vanished, but it will be replaced by right plastic mean bioplastic.

Single-use items, in the form of bottles, packaging, toiletries things, containers, toys and other household things can be disposable and recycled after the use of one time. The mechanism of the degradation of the SUI bioplastic items contributed in the “greener earth theory”.

Biodegradable bags: Using plastic bags is the one of the topiest reasons behind the environmental pollution, as looking at that, it become necessary to find an alternative material. Plastic bags are non-biodegradable which ends up in the landfills and an ocean. The composition of the bioplastic basically derived from natural materials or renewable sources like corn starch, potato starch, cellulose, sugarcane, algae, seaweeds etc. It shows tremendous flexibility, heat resistance, durability and their biodegradability does not show any effect in the environment like petroleum-based plastic bags does. The production rate of poly bags increased day-by-day, non-biodegradable plastics does not fit for this role, biodegradable polymer is the appropriate way to decrease the pollution level. The bio-origin plastic helping to reduce carbon foot printing with the factors like strength, flexibility and barrier properties. Bioplastic can be of two type bio-based and biodegradable.



During the degradation time, biodegradable bioplastic breaks down into organic compounds under suitable conditions which reduces pollution. According to outlook, biodegradable goods help to motivate green nature with the standards by international organization.

2.2 Consumer goods:

Out of the total global production of the plastic,10 million tons of plastic waste has been dumped in the marine world (seas,oceans, rivers). Actions leads to ocean safety now become a serious concern. most of plastic waste includes non-biodegradable material causes harmful and toxic effect towards the sea-life. In the below table, here is the analysis of some goods which showing the comparison between plastic and bioplastic:

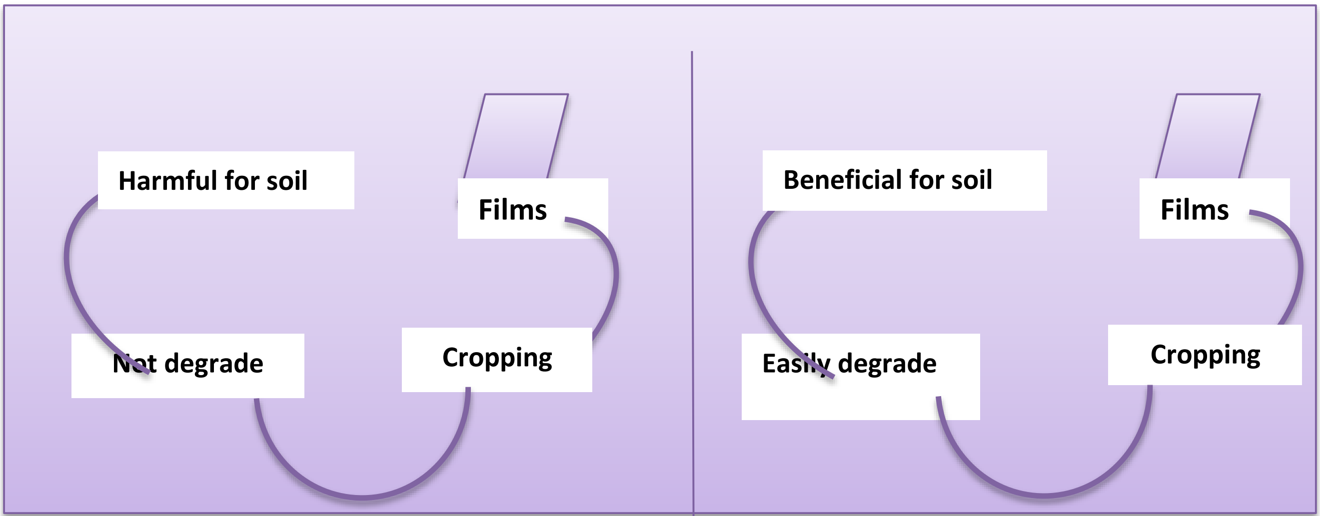
FACTORS	CUTLERY AND UTENSILS		ELECTRONICS CASINGS		TEXTILES	
DEGRADING TIME	PLASTIC	BIOPLASTIC	PLASTIC	BIOPLASTIC	PLASTIC	BIOPLASTIC
	Very low	high	Very low	high	Very low	High
RECYCLED OR DISPOSAL	Hardly	easily	hardly	easily	Hardly	Easily
WASTE SOURCE	13-15%	More than 60%	10-11%	50%	Upto10%	Upto45-50%
PRICE	low	moderate	moderate	high	moderate	High
PRODUCTION RATE	50-60%	30-40%	More than 40%	15-20%	Below 65%	15-19%

CONTRIBUTED IN LANDFILLS	Very high	Very low	high	low	high	Very low
SUSTAINABLITY	Not at all	Strongly yes	no	yes	no	Yes
REDUCE CARBON FOOTPRINT	No	yes	no	yes	no	yes

2.3 Agriculture and Horticulture

Mulching films:

Increasing in the global population definitely increases their needs. To fulfill their necessities, researcher developing innovative and creative ideas with respecting towards environment. Bioplastic mulches films is one of these ideas. when the "bio" word come, it is automatically related with bio-origin, biodegradable, biocompatible.[30] According to study, bioplastic mulches films very effective for the soil quality. most of the petroleum-based plastic are hydrophobic which means they are not easily dissolved or soluble in water that factor indicate that they are not going to degrade easily by the action of bacteria, fungi or other micro-organism.[31] For that reason, bioplastic mulches films are used which easily metabolize in the organic compounds with the help of bacteria like candida, penicillium, thielavia, geotrichun stc.[32] Using protein-based bioplastic mulches film improving the soil structure, crop/plant growth, maintaining the nutrient cycle of the soil, water ratio, soil temperature etc. biocompatibility factors of the mulche films only on depended on the bioplastic material with soil factors. with mulches films, enhancing the agro ecosystem sustainability by reducing the impact of weeds, it is beneficial maintaining the production of fruits, vegetables and crops.[33] The representation difference between plastic and bioplastic mulches films are shown in the below table:



Plastic mulches films **bioplastic mulches films**

Plant pots and trays:

Bioplastics for plant pots and trays are an innovative and eco-friendly alternative to traditional plastic containers. These biodegradable materials are made from renewable plant-based sources, such as cornstarch, sugarcane, or potato starch, and offer several advantages when used in gardening and horticulture applications. When these plant pots and trays reach the end of their life cycle, they can be composted, returning nutrients to the soil. This is a significant advantage for eco-conscious gardeners. One of the primary advantages of bioplastics is their reduced environmental impact. They are biodegradable and compostable. While some bioplastics are designed to resist moisture, they may not be as resilient as traditional plastics. This is something to consider when choosing bioplastic plant pots and trays, especially for long-term use. The range of bioplastic plant pots and trays available in the market may be more limited compared to traditional plastic options and they are slightly more expensive than traditional plastics due to the cost of producing bioplastic materials. However, as technology improves and demand increases, prices may become more competitive. Bioplastics have the potential to be a more sustainable option for plant pots and trays, offering biodegradability, renewable resources, and a reduced carbon footprint. However, their durability, cost, and compatibility with specific gardening needs should be considered. As the technology and infrastructure for bioplastics continue to evolve, they may become an even more attractive choice for environmentally conscious gardeners.

Greenhouse gasses:

Bioplastics decrease the dependency on fossil fuels, which are a significant source of greenhouse gases. Bioplastics can help mitigate GHGs by sequestering carbon during their growth phase. Plants used for bioplastic production absorb CO_2 from the atmosphere, reducing overall emissions.[34] Bioplastic production processes generally emit fewer GHGs compared to the production of conventional plastics. The lower emissions are attributed to factors such as reduced energy consumption and the use of agricultural waste as feedstock. Additionally, some bioplastics are biodegradable, further reducing emissions during disposal. While bioplastics hold promise in reducing GHG emissions, they are not without challenges. One concern is the competition between food and bioplastic crops, which can lead to ethical and sustainability issues. The adoption of bioplastics can encourage sustainable agricultural practices for feedstock production, which may include reduced pesticide and fertilizer use. Sustainable practices can help protect ecosystems and reduce the carbon footprint associated with agriculture. Researchers are actively developing bioplastics that aim to be carbon-neutral or even carbon-negative over their entire life cycle. These innovative materials seek to sequester more carbon than they emit, contributing to a net reduction in greenhouse gas emissions.

Reuse and recycling of bioplastic:

3.1. Mechanical recycling

Mechanical recycling is one of the easiest and cheap processes of recycling; it works with the principle of reduction in quantity. Mechanical recycling is not yet available for commercial use but reducing the molecular weight and tensile strength recycling of biopolymer like PLA and PHA is possible. It is predicted that in order for a given biopolymer packaging and postconsumer mechanical recycling plant to be cost-efficient, there must be at least 200 kT of biopolymer produced worldwide.

Additionally, the recycling facility must be capable of processing at least 5-18 kT yearly. The quality of recycled polymers has been defined as the combination between the degree of mixing, the degree of degradation and the presence of low molecular weight compounds. The assessment of the degradation mechanism is necessary to determine the quality of recycled polymers and guarantee their further performance in second-market applications. When thermoplastic trash is converted back into its original form by primary recycling of plastics, one type of uncontaminated material is used, resulting in a recycled product with similar chemical and physical properties to the original. To improve the quality of the recycled polymers, virgin polymers are frequently combined with recyclates. Mechanically recycled plastic often has a lesser environmental impact than virgin plastic. PW is mechanically recycled into secondary plastic polymers. To create recycled material that can replace virgin polymer, a multistep process that typically involves gathering, categorizing, heat treatment with reforming, re-compounding with additives, and molding operations. Mechanical recycling has inherent limitations, therefore increasing the proportion of post-consumer plastic recycled can help reduce the amount of plastic lost to the environment. Currently, only 9% of post-consumer plastic is recycled.

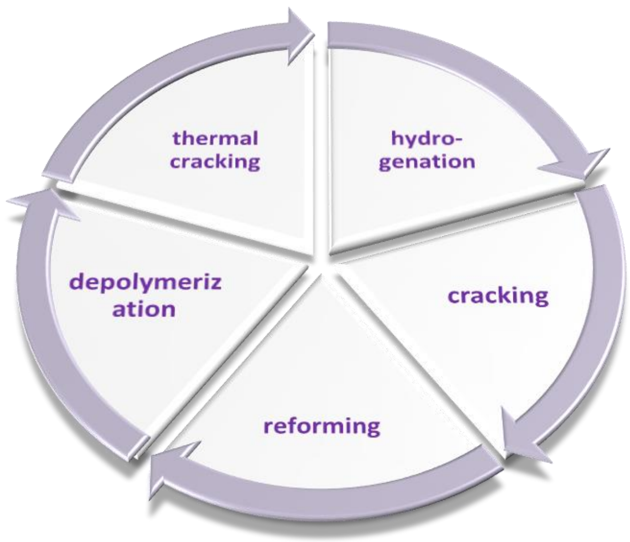
3.2 Chemical recycling:

In contrast to mechanical recycling, chemical recycling offers the potential for making high-quality polymers from waste. Pigment and impurities can also be eliminated. Plastic wastes are transformed into products with lower molecular weight through a process known as the tertiary process. This is a method convert trash to chemicals. Numerous methods, including hydrolysis, glycolysis, thermolysis, solvolysis, alcoholysis and others, are used in chemical recycling. When we consider that this method can recover monomers or other important molecules, it is seen to be both economically and environmentally viable.

Taking the example of PLA, Getting chemicals from thermal or solvolytic depolymerization operations is the method of chemically recycling PLA. High temperatures are necessary for thermal breakdown, which yields a complicated variety of compounds. Alcoholysis allows the creation of lactate esters, but solvolytic depolymerization produces lactic acid, which makes it the preferred method. Chemicals and energy can be recovered by chemical recycling. A variety of gases, liquid hydrocarbons, waxes, and solid particles can be generated during the thermal breakdown of polymers in an inert atmosphere at moderate temperatures. It is possible to recover paraffins, aromatics, iso-paraffins, olefins, and other organic compounds for use as fuels or as raw materials in a variety of industries. Anther example like PHB, PHB can also be depolymerized in the liquid phase by a process called solvolysis, in which a catalyst or high temperatures are used to encourage the nucleophile in the solvent.

One can perform thermolysis with or without a catalyst. The benefits of the catalytic process are reduced temperatures, quicker feedstock breakdown, and a more limited fractional makeup of the final products.The primary problem with this is economic efficiency because commercial catalysts are usually not regenerable, have a short life cycle, and are expensive.

No. of different processes are involved in chemical recycling are shown in below table:



3.3 Challenges in recycling:

According to a 2007 study, a polymer needs to have a critical mass in order to be mechanically recyclable. As a result, biopolymers need to either be 100% interchangeable with currently available recycled resins or be accessible in large enough numbers to meet the required critical mass. It can be difficult to recycle plastics that have been combined with bioplastics. The most efficient way to recycle plastic trash, both biodegradable and non-biodegradable, must be determined by taking into account the marketability and affordability of the final products produced by each recycling method. In any event, it is critical to determine the optimal end-of-life paths for each of the most popular bioplastics, as the production of bioplastics is increasing and these materials will continue to interact with traditional plastics for decades to come. The use phase of bio-based plastics is now brief and resembles current disposable practices because they are mostly being utilized to replace single-use plastics. If bio-based plastics have the same disposal and end-of-life issues as polymers derived from fossil fuels, this might be a problem. It would be best to maximize the product's use period. Bioplastics can be recycled alongside polymers derived from fossil fuels if they are converted into recyclable forms, such as bio-polyethylene or bio-PET. The percentage of recyclable bioplastics is quite low, and the majority of uses for recovered biopolymers are in food service ware and packaging. Preventing possible food sources is the most difficult aspect of producing bioplastics. It is possible to fulfil this requirement by using resources other than food. We refer to these as bioplastics of the second generation. The field of bioplastics faces challenges related to functionality, in particular producing bioplastics that perform as well as their petroleum-based counterparts and achieving a truly positive life-cycle evaluation, which means reaching the objective of carbon neutral materials or reducing the use of fossil fuels. And this can be attained by nanotechnology with increased potential property . The most difficult aspect of producing bioplastics is that it has to be done so without disturbing any possible food sources. Using the non-food resources for this purpose can help improve the situation.

The effects of bioplastics and potential environmental issues have not yet been well studied and comprehended. Therefore, more research is required to improve resource efficiency, mitigate environmental issues, and overcome the limitations of the currently accessible sources. Nowadays, glucose obtained from food and vegetable oils remains one of the most important energy sources for the industrialization of PHA synthesis. Hydrocarbons extracted from waste plastics are now only used in lab settings, but further research should be done to increase throughput and profitability. Combining glycerol and other cellulosic sugars, waste by-products from bio refineries, provides a reliable pathway for the sustainable production of PHAs. The main ways to distinguish between plastic and bioplastic are by their production sources or levels of biodegradability. Certain bioplastics, such as bio-PE, bio-PP, bio-PET, and bio-PA, can be made from both biomass and fossil fuels. Recently, researchers have been looking for less expensive ways to produce the other bioplastics.

3.4 Biodegradation and Composting:

The processes of fermentation by bacteria and metabolic synthesis that transform polymers to CO_2, H_2O and other inorganic chemicals through multiple identified organisms are referred to as biodegradation and composting. Numerous research investigations have been carried out to examine the biodegradability of bioplastics in a number of environmental circumstances, including soil, compost, marine habitats, and other waterways. PLA goes through two phases of biodegradation: first, it is hydrolyzed or oxidised into monomers and oligomers, and then it gets processed by microbes to produce CO2 and H2O. In order to lower its molecular weight, PLA is chemically hydrolyzed in thermophilic environments during deterioration. Because of their great microbial diversity, both compost and soil were primarily considered among these factors. It has been demonstrated that PLA, one of the recent materials to be developed for use in organic food packaging, such as bags, containers, and films, breaks down in composting environment. Only warm, humid conditions with the right microbes can cause PLA break down in compost. The first significant limiting factor is temperature, as the chains' enhanced flexibility merely makes them more accessible to chemical and biological breakdown above the PLA glass transition temperature of 55 °C. Thus, PLA does not break down at mesophilic temperatures (25 and 37 °C), but it does break down to 90% in 120 days at 60 °C. PLA is therefore inappropriate for breakdown in home composting. The results of differential scanning calorimetry (DSC) show that the melting temperatures of PLA-based samples decreased with the duration of the biodegradation process and the disappearance of Tc, confirming degradation and an increase in the proportion of crystallisation that leads to a decrease in Tg. Bio-based materials can be mixed with organic waste and processed in composting plants if composting potential is found. For some applications where organic matter and plastic cannot be separated, such as food waste collection bags, composting is of great benefit and it is important to keep organisms alive in composting plants. In the case of PHAs ,it becoming more and more well-liked in the environmental sector due to their simple disposal. PHAs biodegrade little to not at all in home composting situations with low temperatures or low pH levels. In industrial composting, higher temperatures promote biodegradation. the breakdown of PHAs after 152 days at temperatures ranging from 8 to 30 °C. Degradation of different types of bioplastic at different conditions are shown in below table:

Type of bioplastic	Temperature	Days of degradation	Reference
PLA	58˚C	60	
PLA (powdered)	25˚C	28	
PHB	58˚C	110	
PHA	35˚C	60	
PHBV	21˚C	49	
Starch-based	58˚C	60	
Cellulose-based	58˚C	154	

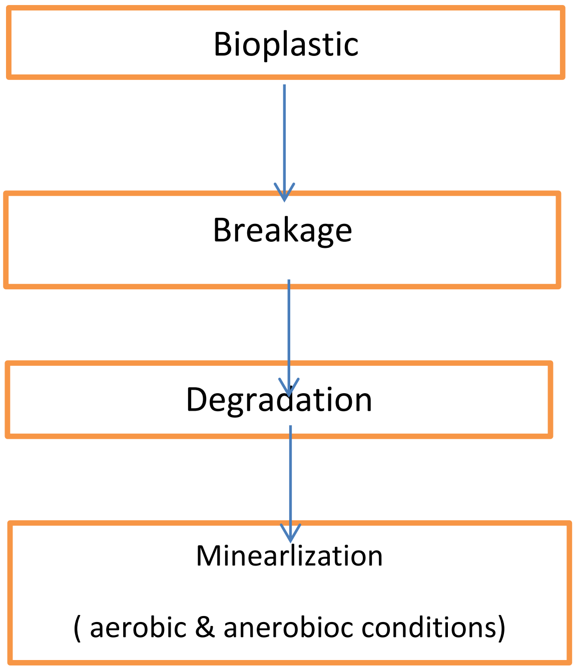
Aerobic biodegradation of bioplastic:

The aerobic biodegradation of biopolymers is an ecologically harmless process that speeds up the breakdown of mixed organic waste through the enzyme-mediated metabolism of a various microbial population in a humid and warm atmosphere under carefully controlled conditions. Microorganisms use the polymer as a source of carbon and energy in an environment with high oxygen levels. They also create carbon dioxide and water as the primary breakdown byproducts, along with the leftover material that is known as compost (Kale et,al.,) Many organisms have the ability to function with oxygen during aerobic breakdown by bacteria, and the end products are CO2 and CH4. Lab settings, origin, and nature all have a major impact on the biodegradation efficiency of aerobic bacteria.

4.1 biodegradation mechanisms:

Enzymatic breakdown

The reason behind the difficulty to decompose the cellulose is the presence of β-glycosidic linkages.[36] Enzymes help to degrade cellulose whose having α-glucosidase like *laetisaria arvalis* is very useful in the degradation of cellulose because it having LPMOs and hydrolytic enzymes. In the case of PLA degradation ,researchers says there are three ways involves : direct isolation ,observation & selection process and enhancement process. PLA can be degrade with the help of enzymes by different reaction like hydrolysis reaction.[37] During the degradation of bioplastic , residues are not toxic, even other organisms can obtain them. Microorganisms produces enzymes which depolymerize the materials. Starch based bioplastic is the general bioplastic which is water soluble polymer. By the blending process of starch with polyvinyl alcohol and polyester degradation rate can be increased.[35] It will higher if the starch concentration become increased because it is biodegradable. Amycolatopis, thermomactimyces and bacillus species are found in soil which play crucial role in bioplastic degradation in soil. PLA degrade about in 12 weeks ,cellulose-based bioplastic take more than 95 days and starch-based bioplastic easily degrade. However, these process help to increase the soil fertility like PHA’s enrich the soil more by increasing microbial numbers in the soil. In the below flow-chart there is the simple way to understand the machanisms of degradation:



Microbial degradation

It is widely known that bacteria are essential to several biodegradation processes. According to current research on PLA-degrading bacteria, the majority of these bacteria mostly belong to the members of the Actinobacteria family.[39] Microorganisms that are capable of digesting polylactic acid are scarce because fewer PLA degraders have been identified than other plastic-degrading microbes. Actinobacteria are one of the rare types of microbes that can break down PLA. The most prevalent actinobacteria that break down PLA are those belonging to the genus Amycolatopsis, specifically Amycolatopsis sp. HT-32. Degradation is a type of decomposition that ends when enzymes, humidity, warm temperatures, and other environmental factors fracture polymers, weakening their chains in the process. Different biodegradation paths can be followed by biodegradable plastics; some, like PHAs, can be broken down by microbes directly, while the breakdown of other types of plastics is aided and enhanced by environmental elements like temperatue, air, and UV rays.[38] A further method of measuring a product's biodegradability is the reduction of its total carbon, or TC. The macromolecular chains break when microorganisms like bacteria and fungi the biodegradation process. Due to the unique characteristics of the microorganisms involved and their increased activity in the soil, this process occurs under various circumstances. Furthermore, the degradation process may be significantly impacted by the properties of the polymer, including chain mobility, crystallinity, molecular weight, type of functional groups, type of substituents present in the polymer structure, and presence of plasticizers and/o additives. PHB is created in combination with the amount of PHB accumulated inside the bacterial cells using specific natural isolates and recombinant bacterial strains on a variety of substrates. In order to optimise the benefits and commercial production of this biopolymer, the most PHA-producing bacterial strain that can grow simultaneously with effective fermentation and a straightforward recovery method must be chosen. However, PHAs' films enrich the soil more than PLA's during composting operations because they boost the soil's microbial population.[40] Fungi were found to have significantly increased along with mesophilic aerobic bacteria and Clostridia species.

Undoubtedly, these alterations were brought about by the quick breakdown of the protein-based bioplastic, which allowed for the release of nitrogen and carbon molecules that fed the microbes and expanded their number.[41]

4.2. Factors Affecting Bioplastics Degradation

Temperature and humidity

Environment factors that affect polymer degradation include humidity, temperature, pH, heat, water, and other culture conditions. They also have a significant impact on the microbial population and enzyme activity. The breakdown of polymers is influenced by temperature and degradation cannot occur in the absence of the necessary temperature. Temperature affects both the rate of the hydrolysis reaction and microbial activity; nevertheless, extremely high temperatures decrease or even completely stop microbial activity. When the temperature raised over the glass transition temperature, the progress of the hydrolysis reaction and the biodegradation of the polymer accelerated quickly. However, as humidity increased, the rate of degradation increased dramatically. As a result, the polymer will break down more quickly in wet environments than in dry ones. Above the glass transition temperature, PLA degrades far more quickly because to the polymer chains' improved adaptability and increased absorption of water, which speeds up both hydrolysis and microbial adhesion. PLA hydrolysis proceeds quickly at temperatures at or above Tg which is 55-66 degree C and high relative humidity levels (>60%).[42] PLA can also experience thermal denaturation when it is molten because hydrolysis can take place with or without minute amounts of water. Due to hydroxide ions catalyze the cleavage of ester groups during hydrolysis ,PLA degrades more quickly in alkaline circumstances. As a result, PLA degradation is accelerated in alkaline environments with high hydroxide ion concentrations. However, as a result of the matrices hydrolysis and enhanced molecular mobility, heating promotes release.[43] But it should be mentioned that heating may eliminate the characteristics of modified polymers and antioxidants, causing a delay in release. Since the enzymes produced by microorganisms become inactive at high temperatures, it is not desirable for polymers to biodegrade.

4.3. Environment Implications;

Microplastic from bioplastic degradation

The plastic with a diameter less than 5 mm is known as microplastics (MPs). Since MPs are small, they are readily swallowed by aquatic and terrestrial creatures, and through the food chain, they can injure humans in an unanticipated way.[45] Currently, one of the main analytical challenges is the quantitative measurement of microplastics.[47] Due to potential interferences with the analyte that could result in negative signal, the complexity of the samples makes analysis challenging. Only very particular circumstances allow for the full biodegradation of bioplastics; these include ideal humidity, the right pH and temperature, a rich environment for microorganisms, and an adequate supply of nutrients.[44] Biofragmentation is one of the first and most crucial stages of breakdown; if the requirements are not fulfilled, microplastics are created in biodegradable polymers far more quickly than in traditional oil-based plastics. The microplastics made of bio polymers have the potential to biodegrade, but they may also have a very long lifespan.

The biodegradability of polymers is also determined by their molecular weight, functional groups, and crystallinity. Since the crystal structure is less accessible to aging media like water, oxygen, or enzymes, the amorphous area of semi-crystalline polymers is more easily broken down than the crystal section.[46] Algae and microorganisms have the ability to colonize microplastics, generating a new microbial activity and habitat. The surroundings and the new communities are very dissimilar from one another. Since microplastics can be detected far from their anthropogenic sources of emissions and even in isolated locations, they can be linked to serious environmental contamination. Primary and secondary microplastics are the two main types of microplastic. Primary microplastics are the byproducts of plastic dust generated from plastic items and particle pollutants released during industrial processing. They are frequently included in formulas for makeup, sunscreen, nail polish, hair coloring, eye shadow, shower gels, and other personal care items that include abrasives and scrubs.

Secondary microplastics are larger plastic particulate matter that is primarily produced when large plastic debris is broken down into tiny fragments by a combination of mechanical abrasion, chemical photolysis, and physical processes exposed to high levels of solar UV radiation.[48] These materials can enter marine environments directly through rivers, shorelines, and sewage pipes. Various methods are employed to analyze the various forms of microplastic. As an example The limit for particle size detection can also be lowered to 1 and 10 mm, respectively, by employing other Raman and FTIR spectroscopic techniques.

Soil and water contamination risks in bioplastic

Compared to traditional materials, bioplastics have been demonstrated to result in reductions in both nonrenewable energy use and greenhouse gas emissions. Because water may permeate the polymer in amorphous areas, hydrolysis—which breaks chemical bonds—can occur, generating bulk erosion in Biodegradable Plastic Mulches (BdPMs), which is characterized by deterioration beginning from cross sections and resulting in a rapid drop in molecular weight. Because BdPMs promote soil moisture and temperature, they can also improve the microenvironment of the rhizosphere. The rhizosphere's microbiome is crucial to the growth, development, and yield of plants.[49]

The amount and activity of the soil microbiota, or microbial biomass, as well as the breakdown of organic molecules in the soil and the release of nutrients are all closely correlated with the biochemical activity of the soil.[51] Therefore, the impact of (bio)plastics on soil microbiota was explained by both the quantity and activity of microorganisms as well as the activity of the enzymes that these organisms produced.[50] More than 150 different kinds of microbial species, such as fungus, bacteria, and actinomycetes, are found in bioplastic-degrading habitats including soil and compost materials and are capable of breaking down different kinds of bioplastics.

In order to look at how the breakdown of bioplastics affected microbial activity, the nitrogen circulation activity was measured as previously mentioned. The nitrogen circulation activity was examined using bacterial biomass, ammonium oxidation activity, and nitrite oxidation activity. The soil next to the plastics was tested in order to measure the biomass of bacteria and fungi. By using a slow stirring analytical method to extract environmental DNA (eDNA), the amount of bacterial biomass in the soil was measured. Using the plate-counting approach, the amount of fungal biomass in the soil was measured.

Under aerobic conditions, PHAs can break down into carbon dioxide and water in 5-7 weeks; however, in anaerobic situations, the breakdown process is accelerated and methane production is beneficial. Twenty days were needed for PHBV to degrade to about 85% in anaerobic sludge.

Comparative life cycle assessments

In order to look at how the breakdown of bioplastics affected microbial activity, the nitrogen circulation activity was measured as previously mentioned. The nitrogen circulation activity was examined using bacterial biomass, ammonium oxidation activity, and nitrite oxidation activity. The soil next to the plastics was tested in order to measure the biomass of bacteria and fungi. By using a slow stirring analytical method to extract environmental DNA (eDNA), the amount of bacterial biomass in the soil was measured. Using the plate-counting approach, the amount of fungal biomass in the soil was measured. Under aerobic conditions, PHAs can break down into carbon dioxide and water in 5-7 weeks; however, in anaerobic situations, the breakdown process is accelerated and methane production is beneficial. Twenty days were needed for PHBV (8% 3HV) to degrade to about 85% in anaerobic sludge.

It is feasible to take into account the environmental effects of a product's usage, repurposing, and final disposal by calculating the downstream consequences of the product during use and at the end of its life. It is necessary to establish an index of overall environmental impact where all indices can be included and fairly weighed because the analysis of LCA data consistently demonstrates that bioplastics have some environmental impact indices lower than those of other traditional plastics, while other indices are in favour of the latter. Biodegradable plastics have been suggested as a way to reduce environmental impacts at end-of-life (EoL).

The breakdown of a material by microorganisms that produces environmentally friendly compounds including CO2, water, and biomass is known as biodegradation. The first step in the three-step process is called biodeterioration, or the "lag phase," in which microorganisms grow inside or on the material and alter its mechanical, chemical, and physical properties.

A technique for measuring the environmental effects that arise along a product or service's whole value chain is called life cycle assessment, or LCA. A full life cycle assessment (LCA), as opposed to the more common carbon footprinting, computes the broader environmental consequences in relation to several impact categories, giving a comprehensive picture of a product's environmental efficiency.[52] Given that whey plastic production is still in its early stages and commercialization has not yet occurred, we decided to compare cradle-to-gate analysis using the LCA approach. A clear definition of the regulations, a scientific discussion of the methodology, the impact assessment technique employed, and the openness of each unique LCI data set are all necessary to guarantee the high caliber of LCA research.[53] This study's LCI takes process-based data into account. The Life Cycle Assessment concept, which is based on cradle-to-grave evaluation, was followed in the development of this study. The phases of the life cycle of petroleum-based plastic and bioplastic boxes made of sugarcane and cassava.LCA is defined by the International Organization for Standardization (ISO) in ISO-14040, along with its uses. According to ISO definitions, the LCAframework comprises an iterative procedure for: Objective and Scope Definition, Inventory Analysis, Impact Analysis, and Interpretation are the first four steps.[54] This Life Cycle Assessment (LCA) set out to assess nine impact categories (eutrophication, acidification, global warming, ozone depletion, photochemical oxidation, human health carcinogenic, human health non-carcinogenic, and respiratory effect) derived from the US EPA's Waste Reduction Model (WARM) and the Tool for Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI 2.1 V1.01). Cumulative Energy Demand was replaced with the TRACI impact category fossil fuel consumption to account for all non-renewable and renewable resources. The normalized effects of five alternative waste management scenarios for bioplastics and food waste were assessed and compared using the CED.[55]

Future prospects and considerations:

5.1. Advancement in bioplastic technology

Enhanced material properties:

According to studies, adding 5% sodium montmorillonite to thermoplastic starch (TPS) boosted its tensile strength from 2.6 to 3.3 MPa and its percentage elongation from 47 to 57%.[56] The new class of bioplastics' considerable increases in shear modulus and Young's modulus were made possible by the use of nanoclay in their development. Combining a polysaccharide with a protein resulted in reduced permeability and increased barrier qualities. Films made of starch and gelatin, starch/alginate and milk proteins, and whey protein-based films with pectin or alginate were among them.[57]

The characteristics of natural fiber-based and biodegradable polyester (PBAT, PLA, PHBV, and PCL)-based biocomposites. Indeed, the biodegradable polyester's and natural fibres' comparable extremely hydrophilic properties led to an increase in the reinforced matrices' tensile and flexural strengths. In the case of alkali-treated fibres, it was noted that the addition of natural fibres increased the thermal stability of biopolymers.[58] According to recent research, one of the raw materials with a great deal of promise for novel bioplastics is soybean oil (SO). In many applications, such as plastic sheet-molding compounds, coatings, and adhesives, modified SO is a promising substitute since, in essence, it improves the qualities of bioplastic.

The properties of the biocomposite may be enhanced by the interaction between the fibre particles and the polymer matrix.In order to investigate the enhancement of the generated bioplastic characteristics, cellulose filler was applied. The primary role in improving the outcome of keratin bioplastic was supported by the interactions between keratin and the Microcrystalline cellulose (MCC) film matrix.

Novel feedstock sources:

carbon-based compounds, as the majority of important chemicals and polymers rely on a carbon source. The chemical industry must shift to using well-defined biomass fractions or heterogeneous biomass sources if fossil fuels are no longer accessible, economical, or politically acceptable. (sugars, starch, cellulose, vegetable oils, etc.).

Starch makes up eighty percent of the calories in our diet; at about $0.5/kg, it is the least expensive processed food item and the second most important agricultural commodity after cellulose. Polysaccharides are a component of seaweeds that are used to make bioplastics.
Agar, alginate, floridean starch, and carrageenan are a few of the polysaccharides found in seaweeds. Commodity bioplastics currently compete with food supply since they are generated from terrestrial crops. Since microalgae use CO2 for photosynthesis and transform it into other forms during central metabolism, using them as a platform for the creation of bioplastics could be a direct way to capture carbon dioxide from the atmosphere. The atmospheric carbon is immediately captured as a polymer if the biomass from microalgae is transformed into bioplastics. An estimate states that for every tonne of polymer produced, three tonnes of glucose must be utilised.

The carbon source is the primary cost-incurring factor and the most important prerequisite for the effective manufacture of PHA. Furthermore, different pre-treatments are needed because the less expensive feedstocks may be poisonous and prevent the growth of other species. For the manufacturing of PHA, the feedstock is conventionally pre-treated. Pre-treatment costs are reduced when wastes are used directly for production.

Blends and Composites:

The growing flexibility of bio-based resources in the creation of compostable and biodegradable polymer systems, particularly the way that two- and three-component polymer blends have been studied to raise the technical appeal and economic viability of this significant class of plastic materials. PBS-related polymers mix in well with PLA and exhibit enhanced qualities.[60]

Reactive blends, which can be made by adding a reactive third component with the right functional groups or a catalyst, can encourage chemical interactions between the two polymers and improve the interaction between PLA and other biodegradable polymers. Increased interfacial adhesion enhances the blends' intended material qualities. The compatibilizers are grafted onto PLA chains to form starch or a third component like PCL. They function as cross-linking agents, chemically binding PLA and starch molecules together. Since starch is typically a minor component in a PLA/starch blend, starch modification is another option to improve the compatibility of starch and PLA. This method is more cost-effective than PLA modification.[61]

The creation of an amorphous starch network and crystallinity, which are substantially impacted by the processing conditions, the concentrations of the plasticizer, and the starch source, are related to the final qualities of starch-based plastics, according to prior studies. A number of scientists have effectively created composite materials, including wood flour, using thermoplastic polymers and cellulose–lignin products.[62] While cellulose fibres added to plastic composites have demonstrated a host of advantageous characteristics, issues with fibre dispersion and fiber-matrix compatibility still exist.

5.2. Policy and Regulation:

Labelling standards for bioplastic:

This guidance document outlines objectives and a plan of action for enhancing the sustainability of bioplastics, which are defined as polymers that have 100% of their carbon coming from renewable forestry and agricultural resources. Many of the concepts apply to other biobased materials as well, even though the focus of these Guidelines is on biobased plastic substitutes for polymers derived from fossil fuels.[64] Procedures and specifications for the identification and labelling of plastics and items made of plastic that are appropriate for recovery through aerobic composting are outlined in ISO 17088.

The purpose of this specification is to set forth the standards for the proper labelling of plastic materials and goods, including plastic-based packaging, as "biodegradable during composting" or "compostable in municipal and industrial composting facilities." Poor levels of compliance from the consumer and activities such as illegal dumping, burying and burning of plastics by the consumer instead of choosing to recycle. The degradation and contamination of the recycling waste stream are caused by consumer confusion and misunderstanding. When selecting environmentally responsible disposal choices, consumers become frustrated due to the lack of an efficient and user-friendly recycling system.

The American Society for Testing and Materials (ASTM), the European Committee for Standardisation (CEN), and the International Organisation for Standardisation (ISO) are the primary standardisation agencies engaged. the primary ISO and CEN standards in use, many of which are similar since CEN standards frequently draw inspiration from ISO standards.[65] In specifically, a polymer must meet the worldwide ISO 17088 or the European EN 13432 or EN 14995 requirements in order to be marketed as biodegradable or compostable. A material is deemed biodegradable in aquatic settings if it completely degrades in 56 days, and in soil if it does so in two years, according to the currently used international criteria for biodegradation (ISO and ASTM).[66]

Extended producer responsibility (EPR) of bioplastic:

EPR, or extended producer responsibility, is another policy tool used to reduce the hazards related to waste management. Through EPR, producers who are both financially and legally responsible can reduce the environmental impact of their products at every stage of their lifespan. The Extended Producer Responsibility (EPR) principle, which extends the producer's liability to the product's post-consumer phase, aims to reduce the release of plastic trash. EPR are generally ineffective for packaging innovation, eco-design, and design advancements and technical advancements. Germany launched the first EPR program in 1991 with the German Packaging Ordinance. Producers of all packaged goods must comply with this ordinance by either joining the industry packaging waste management group Duales Systems Deutschland (DSD) or individually returning their packaging.[67] The Extended Producer Responsibility (EPR) idea is a comprehensive approach that requires producers to take financial, as well as frequently organizational, accountability for gathering, sorting, and processing the products they make as they near the end of their useful life.There are over 400 EPR programs in use worldwide, most of them are located in EU member states where waste legislation establishes fundamental rules for their use.[69]

Financially speaking, one of the objectives of the 2018 EU rule supporting sustainable investment in this direction is to provide explicit standards for the evaluation of green investment funds. Europe uses the extended producer responsibility (EPR) idea to levy levies in order to discourage the manufacturing of plastic.[68] In fact, by reducing the negative effects that plastic items have on society, the environment, health, and safety, EPR can aid in the prevention and mitigation of plastic pollution.

5.3. Consumer Awareness and Behavior:

Role of education in proper disposal

Understanding bioplastics is challenging because the term refers to a type of plastic that is either biobased or biodegradable; the important distinction is that the substance must be one of these, not necessarily both.[70] Not everything biodegrades in the same way. For instance, because PLA breaks down by abiotic hydrolysis and needs certain, higher temperature conditions in compost, it is better described as technically industrially compostable rather than biodegradable.[71] In order to comprehend user views and concerns regarding bioplastics, a variety of statistical methods, including frequency and descriptive analysis, were employed.
Furthermore, Chi-square tests were employed to evaluate the relationships among independent variables, including age, gender, and educational attainment, while taking into account the variables in the nominal scale (categorical) that was utilized in earlier research. Studies showed that most people worldwide have positive opinions on bioplastics.[72] Although there is significant ignorance regarding the proper disposal of bioplastics, breakdown rates, restricted biodegradability, and quality of bioplastics, citizens believe that bioplastics have a more favorable environmental impact than traditional plastics. Food packaging, biodegradable containers for biowaste, and dinnerware are examples of biodegradable materials that can be mixed with organic waste in specialized anaerobic digestion or industrial composting facilities. Thus, it is possible to remove biodegradable plastics from landfills and convert them into compost or biogas. Unless they can be recycled or reused, bioplastics can also be utilised to produce renewable energy. Microorganisms are used in composting to break down organic materials in an aerobic environment. When the composted product is applied to land, carbon is sequestered. The breakdown of food waste and PLA by microbes results in fugitive CH_4 emissions from compost. Composting produces biogenic CO_2 emissions, which are not included in calculations of global warming potential Bioplastics were created as possible replacements for plastics derived from fossil fuels; nevertheless, in order to give precise and thorough answers on the relative sustainability of bioplastics and fossil fuel-based plastics, a thorough analysis of their effects on the environment and society is necessary. PLA and the starch-based bioplastic with PET and PE have been compared through life cycle analysis (LCA) in order to analyse the optimal final disposal of bioplastic waste to maximise energy savings. The results show that, in terms of global warming potential, PLA is less impactful than −60% and −35% compared to PET and PE, while the starch-based bioplastic results in a reduction in GHG emissions of approximately −45% and −20% compared to PET and PE.

Importance of reducing consumption

Composting, an expedited and regulated solid state fermentation at a high temperature that turns trash into "compost," a stabilised material that resembles humus, is one method of recycling biowaste. Anaerobic digestion is another biological waste treatment method that yields compost and biogas as its end products. Farmers' tasks are made simpler by biodegradable mulch films, which do not require removal from the soil or disposal due to their biodegradation and lack of toxicity or accumulating effects. Mulch films made of starch have been specially formulated for crops that take one to nine months to mature, and they can be processed similarly to conventional polymers. Certain biodegradable polymers can only break down under certain precise and tried-and-true circumstances at particular active dump sites. Methane gas, which is produced during decomposition during composting, is a greenhouse gas that is many times more potent than carbon dioxide. Since biodegradation is accelerated at higher temperatures and in the presence of fungi, which are only present in compost and soil environments, it proceeds more quickly in compost, fresh water, landfills, and soil. The rate of biodegradation is highly dependent on the degradation technique and environment. It's crucial to remember that biodegradable plastics are a subset of compostable plastics. Under composting conditions, biodegradable plastics (such PLA and TPS) may take longer to break down than compostable plastics, which break down more quickly. While recycling is higher on the waste management hierarchy, incinerating plastic and bioplastic waste with energy recovery can be helpful in getting rid of all the non-recyclable and non-biodegradable plastic trash, and it is unquestionably better than landfilling. The quantitative analysis's findings imply that the environmental effects of conventional and bio-based plastics are highly variable and reliant on the specific study assumptions. To really grasp where the opportunity for biodegradable substitution resides, it is critical to acknowledge both the benefits and drawbacks of BBPs. Promoting BBPs should concentrate on plastic packaging in areas where efficient recycling strategies aren't working.

Conclusion:

Globally, bioplatics has developed into a cutting-edge field of study for scientists. The necessity for environmentally friendly alternatives to materials derived from fossil fuel sources has propelled this forward-thinking development. Given the benefits of bioplastics, there are undoubtedly enough of materials and resources available to develop and discover new applications for bioplastic. It has been found that because of their distinctive layered structure, natural abundance, and accessibility, clay nanoparticles offer an inexpensive and adaptable raw material for starch-based nanocomposites. Additionally, the biotechnology of microorganisms presents a chance for the production of bioplastics, as it has the potential to be highly applicable and commercializable across a range of industries, including agriculture, medicine, pharmaceuticals, etc. Regulations pertaining to product labelling need to be improved in light of the raw materials used, energy used, and emissions produced during production and use. Policymakers will be able to assess whether using new-generation bioplastics is indeed better for the environment with the aid of these studies.

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