

Optimization of Starch-Based Biofilm Formulation and Biodegradability Assessment

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Abstract

Traditional or regular plastics have become one of common sources of pollution in the environment. Because of non-degradability, it can easily accumulate in environment and releases toxic compounds. These compounds can even enter in to food chain and cause affecting the people health. Due of their negative impact on the environment, various research is going on to derived starch based or biodegradable plastics, which can substitute these regular plastics. Present study aims to optimizing the formulation of starch-based biofilm by adding some additives like glycerol, citric acid and gelatine. Glycerol is used as a plasticizer; citric acid can improve the stability in bioplastic and gelatine is a food material or biopolymer that provides a brittle and elastic nature in bio plastic. This work also addresses the biodegradability on starch-based bio plastic. The biodegradability rate was assessed in the soil, microbial activity and in water at different incubation temperature. 1.5% was maximum degradability shown in the garden soil and incubated at 30°C. In water, maximum degradability was shown in the sewage sample is 3.3% followed by marine and normal water respectively at 30°C. *A. niger* was used for microbial degradability in the bio plastic, it has shown 60-100% of degradation and the biodegradability were examined by ASTM G21 method.

Keywords: Traditional plastics, biodegradability, starch, glycerol, citric acid.

1. INTRODUCTION

In today's world, conventional plastic has become a very generic material, which is used almost everywhere, as for many industries like Pharma, Packaging, Automobiles, Chemical, Transport and many more. In all over the world, millions of synthetic plastics are produced and used in a year [1]. Traditional plastics are made of synthetic polymers, crude oil, petroleum based materials or common gases, which do not breaks easily in the environment for a long time. However, due to non-degradable in nature regular plastic waste gets accumulated in the ocean, on land and can caused issues for living beings and serious ecological harm, such as, soil pollution, water contamination, and so. [2]. In order to reduce the negative effects of plastics, introduction of bio plastic is a boon as it is environment friendly, used similar to regular plastic and after being thrown in to environment it is destroyed by the activity of microorganisms [3]. Bio plastics are generally formulated from starch which is used as an important component of the bio plastics and other organic compounds like glycerol, gelatine and citric acid are used as additives. Starch is a natural, polysaccharide, polymeric carbohydrate consisting of a large number of glucose units linked by a glycosidic bond., potatoes found higher amount of starch, which is easily found to anywhere. Amylose and amylopectin are two types of polymers found in starch; it can be readily extracted from plants and is affordable [4]. The importance of this bio plastic production is to the formulation of an environment friendly material that can be readily degradable in the environment.

This work addresses to the formulation of bio plastic and also checks the biodegradability rate by three different ways in soil, aquatic and ASTM G 21 method, degradability is a natural and chemical process that occurs

due to microorganisms present in the environment and some factors also affect them like (temperature, light, moisture), some organic compounds are released after degradation of bio plastics such as carbon dioxide [5]. The degradability, process can be characterized by such that there is increase in the percentage of weight loss and decrease in the weight of synthesised sheet [6]. In starch-based bio plastics the biodegradability can be assessed by observing some physical and chemical changes, starch-based biofilm are produced simply and it is a non-reactive and non-toxic material that can be most widely used in food packaging.

2. MATERIALS AND METHODOLOGY

Potatoes were obtained from local market for starch extraction, street and garden soils are get from Gautam Buddh Nagar and some chemical reagent, Glycerol (C₃H₈O₃), Gelatine (C₁₀₂H₁₅₁N₃₁O₃₉), Citric Acid (C₆H₈O₇) were obtained from Advantec Life Sciences, India.

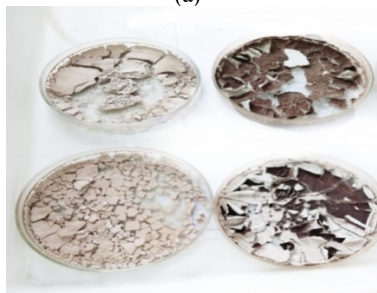
2.1. Starch extraction

The following procedure are used for the isolation of starch from potato, 1 kg of potato must be first clean with fresh water, then remove the skin of potato and again wash with clean water. Cut all the cleaned potato carefully in the small pieces, then grind all the potato pieces in a blender and prepare potato slurry. After that, sulphur dioxide was usually added in to the potato slurry for prevent the potato from turning brown, preventing it from getting oxidised and from spoilage. Starch separated from potato slurry through filtration, the filtered solution or obtained starch were poured in to glass plate and finally placed in oven for drying at 100°C for 2-5 hours, 30-50% of pure starch was obtained in 1kg of potato. Some phytochemical test was also done in extracted potato

starch like moisture content (12.7 %) and pH (6.7). Wet starch or before placed in oven and dried starch after oven are shown in figure 1.



(a)



(b)

Figure 1: (a) shows wet starch samples before dried and (b) are shows extracted dried starch, were heated in the oven for 100°C.

2.2. Bio plastic Preparation

For the synthesis of bio plastic, Starch is the basic compound used in the formulation but this formulation also includes a group of renewable material with various applications and unique properties such as glycerol, gelatine, citric acid. [7] [8]. Bio plastic is formulated by using the following technique:

In 50 mL of distilled water, various components like the starch, gelatine, glycerol, and citric acid were added manually at different proportions. The combination was tenderly blended through glass pole by hand for 5 min and after blended in shaker for 10-15 min at the rate of 150 rpm. At that point the prepared solution was heated on a hot plate stirrer or magnetic stirrer at 100 °C for 50 to 70 min, persistently [9]. Prepared solution was left for cooling at room temperature for few seconds and afterward this semisolid solution was poured on a glass plate or petri plate and spread consistently. Poured semisolid liquid can be dried in different ways like, 2 to 3 days in under sunlight, 4 to 5 hours in oven and 1 day in incubator at 30°C. The obtained bioplastic sheet was formed with various compositions as shown in the Table 1.



Figure 2: solution prepared for bio plastic films

Table 1: Composition of bio plastics.

Sample	Starch	Glycerol	Citric Acid	Gelatine	Distilled Water
S1	5.0gm	1.5gm	0.5gm	1.0gm	50ml
S2	6.0gm	1.5gm	0.5gm	1.0gm	50ml
S3	5.0gm	2.5gm	0.5gm	1.0gm	50ml
S4	5.0gm	3.5gm	0.5gm	1.0gm	50ml
S5	5.0gm	1.5gm	1.5gm	1.0gm	50ml
S6	5.0gm	1.5gm	2.5gm	1.0gm	50ml
S7	5.0gm	1.5gm	0.5gm	2.0gm	50ml
S8	5.0gm	1.5gm	0.5gm	3.0gm	50ml

2.3. Biodegradability test of bio plastic

2.3.1. Soil Test

The obtained bio plastic sheet was cut carefully into (5x5cm) and was placed in two different soil samples which were collected from garden and street side in local area of Greater Noida. Now these buried samples were incubated at different temperature (20, 25 and 30°C) for 25 days and examined in every 5 days. The buried samples were measured carefully before and after placing in incubator, the weight reduction or increase the per cent of biodegradability in bio plastics was determined by given equation (1), W_0 is the initial weight of the sample before reduction or before placed in incubator and W_f is final weight after reduction [10].

$$\%Weight\ loss = \frac{W_0 - W_f}{W_0} \times 100 \quad (1)$$

2.3.2. Water Testing

Prepared Bio plastic sheet were cut in following measurement (5x5cm) and placed on to 50ml of different water samples that are normal, marine and sewage water. Now incubated at different temperature (20,25 and 30°C) for 10 days with determining time in every 2 days. Obtained bio films were weighed before and after placing in incubator, same as soil test. The weight loss and increment of biodegradability in the bio plastics was calculated by using the above equation (1).

Table 2: Properties of sample.

Properties	Sample	Street Soil	Garden soil
<i>PH</i>	6.79	6-7	5-6
<i>Moisture Content</i>	7.9(%)	6.6(%)	11.9(%)

2.3.3. ASTM G 21Method

Products made of plastic are generally tested with the ASTM method but this technique can be used for other materials as well, ASTM G 21 have different version. In this work *Aspergillus niger* is used in ASTM G 21 and this method is used to determine the effect of fungus on obtained bio plastics sheet. *Aspergillus niger* is a very common environmental fungus that is easily found in the contaminated materials. Which can be isolate from plastic waste and soil through serial dilution [11] [12]. Fungus can grow easily on bio plastic because it is a biopolymer and a good source of nutrient [15].

Here, readily available pure culture of *A. Niger* was used to determine the biodegradability rate of synthesised bio

plastic sheet. PDA media was prepared and poured in to sterile petri plate in laminar air flow, after solidification, synthesised bio plastic was placed on to the surface of prepared media followed by spreading of pure culture of A.niger. Petri plates were then placed for 1 to 2 weeks at 30°C. Duplicates were prepared simultaneously for the above process to determine the rate of degradation. Fungus growth in samples can be assessed by degradability means higher fungal growth in sample shows greater degradability in bio plastic. In this method, fungal growth on material can be assessed through scoring.

Table 3: Fungal scoring on bio plastic.

Score	The growth of fungus in per cent (%)	Days
0	0 or no growth	1-2nd
1	Initial or 10 % of growth	3 rd
2	10 to 30 % of growth	3 to 5 th
3	30 to 60 % of growth	5 to 7 th
4	60 to 100 % of growth	2 nd week

3. RESULTS AND DISCUSSION

3.1. Bio plastic samples

Different amounts and combinations of starch and additives were used to synthesize starch composite bio plastics. Total eight bio plastic sheet were obtained which was formulated in different proportion but seventh sheet

were shows the best bio plastic property. Further several testing were carried out on obtained bio plastic sheet to the basis of their biodegradability, including- aquatic or water, microbial and soil testing.

3.2. Degradability of bio plastic using Soil Test

The results of weight loss and degradation rate of bio plastic are represented by graph which is shown in figure 4 below: The rate of biodegradability increases with time, longer the burial time of bio plastic in soil, more will be the degradation. Bio plastics are formulated from starch and other organic material, because of that it can easily degrade in soil and some microorganisms present in soils also help for the degradation. Starch has loose bond between two carbon residue which can be easily degraded, the degradation reaction in soil for biopolymer causes weight loss and chain shortening and the weight reduction of biofilm was due to the organic materials used in bioplastic that is readily digested by microbes [13][14]. The biodegradability rate is higher in garden soil as compare to street soil because the moisture level is higher in garden soil. Biodegradability of bioplastics in both the soil were sampled at every 5 days, in which maximum degradability was found in street soil is 1.1% at temperature 30 °C and 1.5 % of maximum degradability was shown in garden soil after 25 days of burial period at temperature 30°C.



Figure 3: Total obtained bio plastics sheets at different compositions.

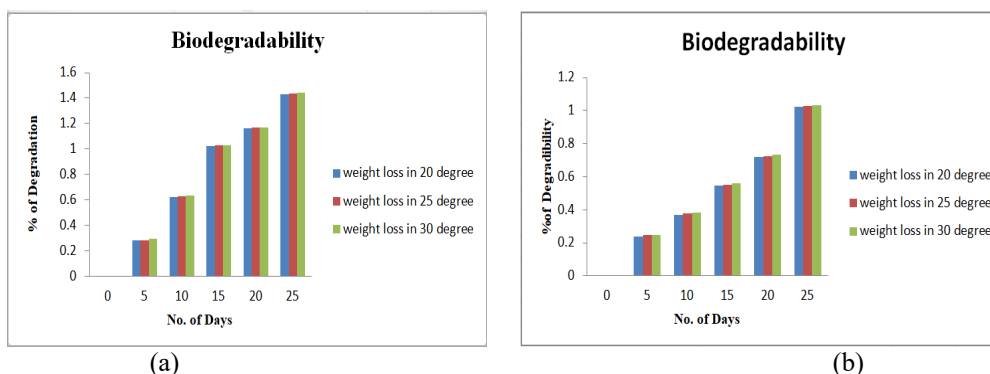


Figure 4: Biodegradability graph of bio plastic on garden and street soil at different temperature. Weight loss of biofilm was increased every day (a) degradability on garden soil and (b) degradability in street soil.

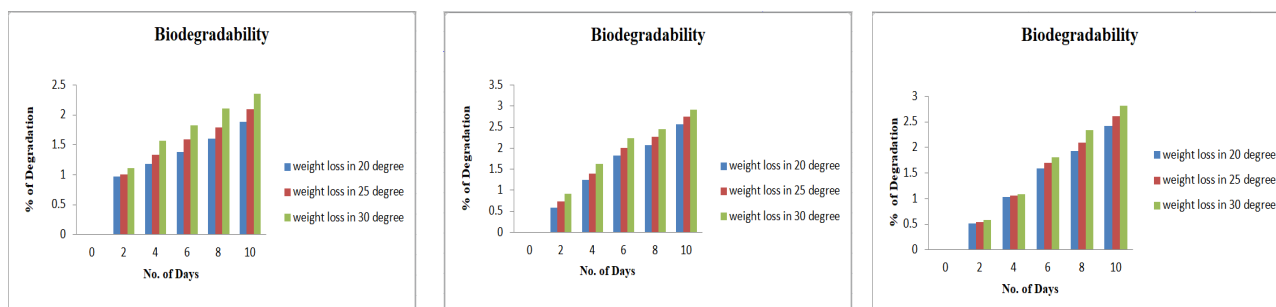


Figure 5: Biodegradability graph of bio plastic increased every day in aquatic environment at different temperature (a) shows in normal water (b) sewage water and (c) marine water.

3.3. Biodegradability of bio plastic in aquatic environment

The water degradability rate of bio plastics is shown in Figure 5. In aquatic environments biodegradation can occur by both aerobic and anaerobic methods. Moreover, materials used to produce bio plastics such as glycerol have great solubility in water. Some environmental factor like oxygen and UV radiation can affect in degradation of bio plastic. This may be the reason that biopolymers are easily degrade in water as compared to synthetic polymers. Degradation rate has been found to be higher in sewage water than other two samples. Biodegradation of bio plastic in aquatic habitats are depends mainly on the environmental conditions. Maximum degradation was shown in sewage water 3.3% at higher temperature and sample followed by marine water 2.9% and normal water 2.8 % are at 30°C.

3.4. Biodegradability of bio plastic using ASTM G21

This method was used for degradability of bio plastic in PDA media. In 24 hours, there was no fungal growth shown on bio plastic sheet and media. The growth of *A.niger* were shown in after 48 hours on the surface of media but still no growth are shown on the surface of bio plastics sheet. *A. niger* growth begins on the media after 3rd day and start covering the whole surface of petri-plate after day 6. The black colour of *A. niger* spores can be seen on bio plastic sample from day 7, the biomass of *Aspergillus niger* on bioplastic and media was increased every day. A proper growth of *A. niger* can be seen in the petri disc on 12th day. The degradability of bio plastic by *A. niger* was time dependent and maximum degradability was found on 12th day.

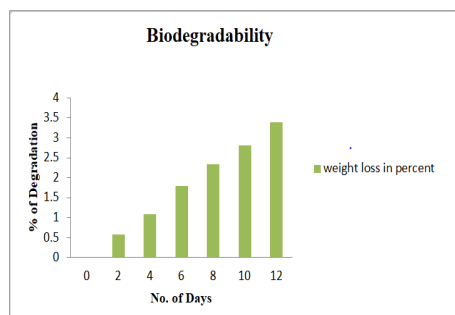


Figure 6: Graph shows the degradability rate of starch based bio plastic increased every day by microbial activity in ASTM method.

4. CONCLUSIONS

The bio films made in this study have shown good biodegradability and thus can be used to produce the carry bags and packaging material. This will reduce the accumulation of plastics in water bodies and drainage systems. As this biofilm is made of starch, after degradation it will not pollute the water bodies and will not be harmful for the life inside sea. At present the cost of biodegradable plastics is more but with increased demand and awareness the cost will eventually reduce. In future antioxidants and antimicrobial agents can also be incorporated in order to enhance the shelf life of packaged foods and the film itself.

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