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Degradation Rate of Microbes on Starch Based Bioplastic: A Quantitative Study

Om Joshi*, Jaswant Kandikanti, Ronit Savale, Himanshu Patil and Manisha Survase
Department of Biotechnology, Institute of Biosciences and Technology
MGM University, Chatrapati Sambhajinagar, India
*Corrosponding author Email: theomjoshi195@gmail.com

***Abstract*— Starch based bioplastic has seen a remarkable growth in replacing synthetic plastic. Bioplastic have similar features as synthetic plastics while providing extra features because of their low carbon footprint. Interest in competitive biodegradable materials is growing to limit environmental pollution and waste management problems. This can be seen as the beginning to replace the single use synthetic plastics with biodegradable starch based plastics and step forward for a greener environment.**

Bioplastics made from starch which is composed of a bio-polymer called Amylose. Amylose is an unbranched linear molecule polymer composed of glycosidic linkages. Starch is a reserve carbohydrate found in the majority of plants. The largest source of starch is corn and rice. In this study corn starch was processed into biodegradable plastic.

Amylose denatures by the action of enzyme Amylase. Amylase is an isoenzyme commonly found in various microorganisms which are present in soil. The degrading activity of the isolated microorganisms on starch based bioplastics was studied and a comparative analysis was made. The degradation rate of the isolated bacteria and fungus were as follows: Cocci shaped bacteria: 5.1 mg/hr, Rod shaped bacteria : 4.5 mg/hr, Cocci shaped + Rod shaped : 2.2 mg/hr. Two fungal species belonging to Aspergillus showed the degradation rate as 2.5 mg/hr and 3.2 mg/hr while their combination showed 3.3 mg/hr.

***Index Terms*— Bioplastic, Starch, Amylose, Amylase, Amylopectin, Hydrolysis, Degradation rate.**

I. INTRODUCTION

Plastics have been playing a vital role in industrial and as well as in household applications. It has now almost dominated every sector, it may be for preparation of plastic toys instead of wooden toys, or bottles, baggages, modules of vehicles, furniture, dresses, etc., [1]. One of the reasons why plastics are so popular is because they are cheap, easily available and perdurable [2]. In totality around 8.3 billion metric tons of plastics has been created till now, while around 6.3 thousand metric tons of plastic waste has been produced. Only 9% of that waste plastic was recycled, 12% incinerated and the leftover 79% is cumulated in the sanitary landfill or in the environment. It is estimated that by 2050, 12 thousand metric tons of wasted plastics will accumulate in the sanitary landfill or in the open environment [3]. Though plastics have multiple advantages it also has concerning disadvantages like it can't be degraded [4], its production requires lots of energy [5] and they are menace for the environment [6]. Due to these disadvantages more sustainable ways are being searched and one of them is use of bioplastics.

A bioplastic can be defined as a polymer that is manufactured into a commercial product from a natural source or renewable resource [7]. Bioplastics may be openly taken out from natural resources like, proteins, lipids, and polysaccharides (e.g., starch, chitin, and cellulose) [8]. Approximately 50% of bioplastics used in today's industry is starch based bioplastic. Its production is simple, and they are widely used for packaging applications [9].

The tensile properties of starch and glycerol which acts as plasticizer makes starch based bioplastic suitable for it used as packaging material and for trade applications eco-friendly polyesters are mixed with starch based bioplastic [10].

Pure starch is white in color. The starch powder does not possess any specific taste or odor. Furthermore, pure starch cannot be dissolved in cold water or alcohol. It is non-toxic, biologically absorbable, and semi-permeable to carbon dioxide. The linear and helical amylose and the branched amylopectin are the two types of molecules present in starch [11]. Most starches contain 10-20 percentage of water soluble amylose and 80-90 percentage of water insoluble amylopectin this ratio can vary depending on the source [12]. Waxy or glutinous starch from corn and other cereals contains little or no amylose, while a sugary mutant corn and some of the legumes contain amylose in greater abundance than amylopectin [13]. In addition to these glucans, small amounts of proteins and lipids are also present in starch [14]. Amylose is straight chain polysaccharides in which α-D-glucose units are joined 1-4. Chain lengths vary from 250 to 350 glucose units, and the long molecules appear to be coiled in α helix. Amylose is soluble in water but forms hydrated micelles. In such micelles the long chain is twisted into a helical coil. The structure of amylase

contributes to the gelling characteristics of cooked and cooled starch [15].

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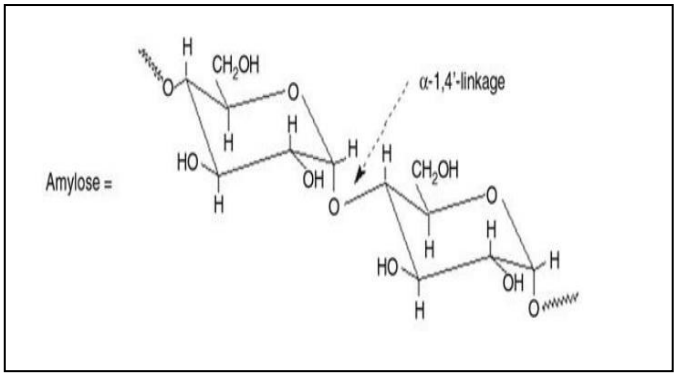

Fig 1. Molecular Structure of Amylose (source Shanta Pokhrel, 2015)

Amylopectin also has a backbone of α- (1→4) linkages but, in addition, the molecule is branched through α- (1→6) linkages to the extent 4-5 percent. The length of the linear unit in amylopectin is about 20-25 percent glucose units. Amylopectin is responsible for the thickened properties of starch preparations but it does not contribute to gel formation [15].

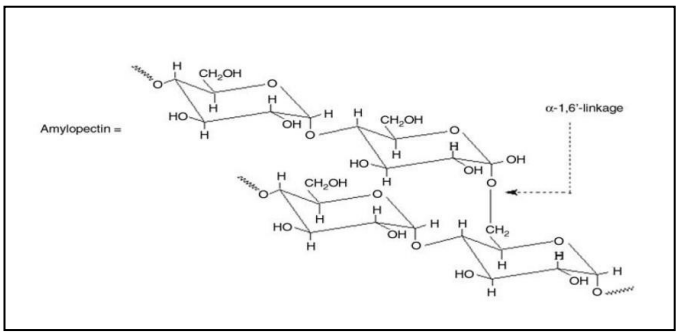

Fig 2. Molecular Structure of Amylopectin (source Shanta Pokhrel, 2015)

Starch, which is the main component of our bioplastic, can be degraded by the enzyme Amylase.α-Amylase (EC 3.2.11) an endo-hydrolases that acts on α-(1,4)-glycosidic linkages in starch and other related oligo and polysaccharides, thus causing the release of maltooligosaccharides and glucose on the α-anomeric form; α-amylases are essential for the conversion of starch into oligosaccharides and are critical for many organisms that use starch as a primary source of energy [16]. Bacteria and fungi tend to secrete amylases outside the cells to perform extracellular digestion of starch into sugars. Increasing industrial demand for microbial amylases has been observed due to their specificity of reaction, mild conditions prerequisite for the reaction, and less energy consumption than the conventional non-enzymatic chemical methods [17]. Bacillus is a common bacterial source for industrial amylase production. Reportedly, Bacillus strains have been extensively used industrially to produce á-amylase including *B. amyloliquefaciens*, *B. subtilis* [18], *B. licheniformis* [19], *B.stearothermophilus* [20] , *B. megaterium* [21]. From Fungal strains it is been reported that *Rhizopus sp*. [22], *Aspergillus fumigatus*, other *Aspergillus* species. [23] and *Corticium rolfsii* [24].

In this study Bioplastic was prepared from sweet corn (*Zea mays*) which is known to produce high amounts of Amylose rather than amylopectin and we isolated bacteria and fungi from soil collected from Dumping yard, Kalyan, Maharashtra, India. The isolated microbes were incubated with bioplastic in a controlled environment in various combinations to calculate the degradation rate of the microbes on starch based bioplastic.

II. METHODOLOGY

The main objective of this study was to prepare bioplastic from sweet corn starch and to check the degradation rate of microbes found in soil on the prepared starch based bioplastic.

A. Production of starch based bioplastic:

25 gm of corn starch was weighed and was mixed with 150 ml of distilled water in a pan. 5 ml acetic acid and 5 ml glycerol were added to the pan and this solution was heated. Heating continued as the solution became thick slurry. It was made sure that the starch was completely dissolved and no chunks of starch were present. Once a thick slurry was formed the slurry was added into the desired molds to give it a shape and dried in sunlight for 24 to 48 hrs.

After 24-48 hours of sun drying desired shaped plastics were obtained.

B. Collection of soil sample:

Soil Sample was collected from Municipal Corporation Dumping yard, Kalyan, Mumbai, Maharashtra, India. Sterile instruments were used to collect the soil sample, it was collected below 15 cm of soil surface.

C. Isolation of microbes from soil sample:

One gm of soil was serially diluted upto 10^{-7} in 9 ml of 0.85% saline solution and 100 ul of each dilution was spread on seven nutrient agar petri plates respectively and were incubated at 37ºC for 24 hours. Classification of newly obtained colonies was done on the basis of shape, size, texture,

color etc.,.. and the observations were noted. Distinguished fungal and bacterial colonies were subcultured on fresh starch agar to obtain pure isolates.

D. Characterisation of isolated microbes:

On the basis of size, shape, and structure of colonies, gram staining and biochemical test like Indole test, Methyl red test and Voges Proskauer test bacterial colonies were classified and fungal colonies were classified based on spores observed under 100x microscope by lactophenol cotton blue staining and colony's morphological characteristics on agar plates

E. Identification of starch degrading microbes:

200 ml of Nutrient agar (bacteria) and 200 ml of Czapek dox agar (fungus) with 1 percent (w/v) starch were prepared and poured on petri plates. A well was punctured into the agar of these plates with help of gel borer and bacterial and fungal isolates were inoculated into these wells and kept for incubation at 37^0C for 48 hrs. Then 1 percent iodine solution was flooded on these plates and were left undisturbed for 5 minutes. Iodine solution was washed clear zones formation was checked. Clear zones indicate hydrolysis of starch by the microbe on the agar plate. Therefore microbes showing clear zones can degrade starch based bioplastic.

F. Degradation rate of microbes on starch based bioplastic:

Inoculation of each microbe in 100 ml of nutrient broth in a conical flask was done with 1 gm of starch bioplastic. These flasks were incubated at 37^0C and 28^0C for bacteria and fungi respectively. After every 24 hours the bioplastic was removed from the flask, dried to remove excessive water and was weighted on pan balance and again resubmerged into the flasks and this process was continued for 7 days. On the basis of observed data degradation rate of each microbe was calculated.

The formula used to calculate degradation rate was:

$$\text{Degradation rate} = \frac{W_A}{24 \text{ hrs}}$$

Where,

W_A = Average/Mean of weight reduced of bioplastic by microbe.

G. Percentage decrease in weight of bioplastic:

After 7 days percentage decrease in weight of bioplastic was calculated to observe how much weight has been reduced by each microbe after 7 days .

Formula used to calculate percentage decrease in weight was :

$$W = \frac{(W_O - W_F)}{W_O} * 100$$

Where,

W_O = Initial weight of bioplastic.

W_F = Final weight of bioplastic.

III. RESULTS AND DISCUSSIONS

A. Production of Starch based bioplastic:

Fig 3. Preparation of Bioplastic

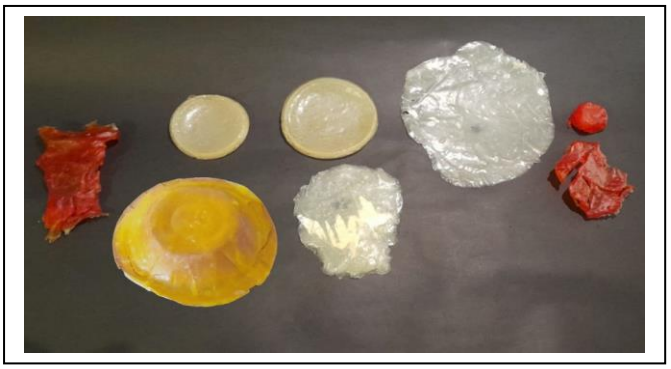

Fig 4. Bioplastic of the desired shape obtained based on the shape of the mould.

B. Isolation of microbes from soil:

In this study, 9 colonies of both fungus and bacteria were isolated out of which 5 morphologically different strains of bacteria and 4 morphologically different strains of fungi were observed.

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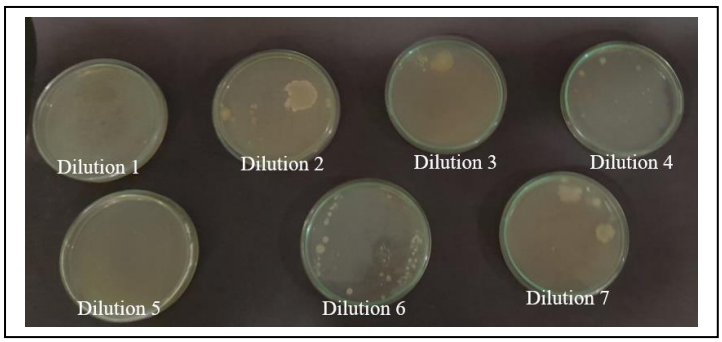
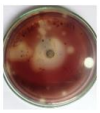
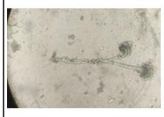
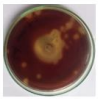
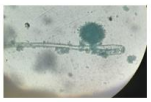


Fig 5: Growth of microbial colonies on nutrient agar plates spread with soil solution after serial dilution visible after 24 hours of incubation.

Fungi	Starch Hydrolysis test	Lactophenol cotton blue staining(100x)	Agar plate
SDF 1			
SDF 2			

C. Identification of starch degrading microbes:

Out of five isolated bacterial colonies, only two colonies showed clear zones and out of four fungal colonies only two colonies showed clear zones. The bacterial and fungal colonies were named as SDB 1 and SDB 2 (Starch Degrading Bacteria) and SDF 1 and SDF 2 (Starch Degrading Fungi).

D. Characterization of isolated microbes:

Under 100X microscopic observation of Gram's staining showed that SDB 1 was gram negative cocci shaped and SDB 2 was gram negative rod shaped. For SDB 1 Indole test, Voges Proskauer test was found to be positive, while negative for Methyl-red test. For SDB 2 Indole test, Methyl red test were positive while Voges Proskauer test was found to be negative. On the basis of observation of spores under 100x microscope by lactophenol cotton blue staining and colony morphologic characteristics on agar plate SDF 1 had similar morphological characteristic to *Aspergillus fumigatus* while SDF 2 had similar morphological characteristic to *Aspergillus niger*.

TABLE I. STARCH HYDROLYSIS AND CHARACTERISTICS OF ISOLATED BACTERIA.

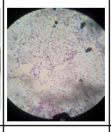
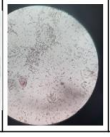
Bacteria	Starch hydrolysis test	Gram's staining (100X)	Indole Test	MR test	VP test
SDB 1					
SDB 2					

TABLE II. STARCH HYDROLYSIS AND CHARACTERISTICS OF ISOLATED FUNGUS.

E. Degradation rate of microbes on Starch based bioplastic:

Three different flasks with nutrient broth were used, flask no. 1 inoculated with SDB flask no. 2 inoculated with SDB 2 and flask no. 3 inoculated with both SDB1 and SDB 2. Same was followed with fungi strains i.e. flask no. 1 inoculated with SDF 1, flask no. 2 inoculated with SDF 2 and the third flask inoculated with both SDF 1 and SDF 2. On day 1, approximately 1 gm of bioplastic was weighed and added into each flask. On day 7 SDB 1 broth had approx. 0.2 gm, SDB 2 had approx. 0.5 gm and their combination had approx. 0.7 gm, SDF 1 and SDF 2 had approx. 0.7 gm and its combination had approx. 0.6 gm.

TABLE III. REDUCTION IN WEIGHT OF BIOPLASTIC BY BACTERIAL STRAINS

Days	SDB 1	SDB 2	SDB 1+2
Day 1	1.018 gm	1.164 gm	1.043 gm
Day 2	0.942 gm	1.103 gm	0.984 gm
Day 3	0.866 gm	0.992 gm	0.971 gm
Day 4	0.717 gm	0.858 gm	0.967 gm
Day 5	0.612 gm	0.777 gm	0.904 gm
Day 6	0.395 gm	0.550 gm	0.826 gm
Day 7	0.272 gm	0.511 gm	0.723 gm

GRAPH I. GRAPHICAL REPRESENTATION OF TABLE III

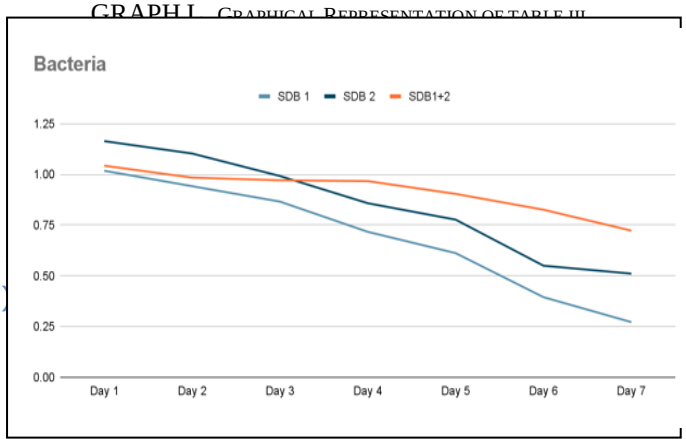
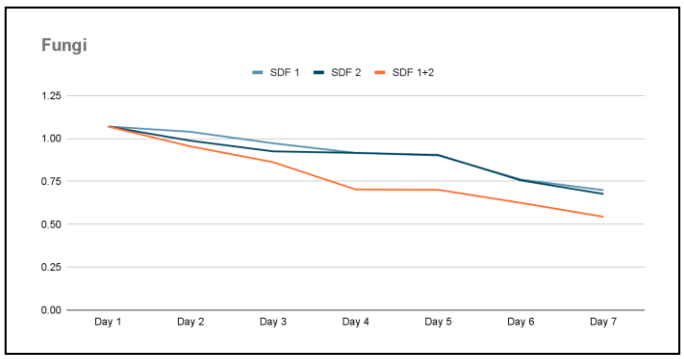


TABLE IV. REDUCTION IN WEIGHT OF BIOPLASTIC BY FUNGAL STRAINS

Days	SDF 1	SDF 2	SDF 1+2
Day 1	1.070 gm	1.070 gm	1.070 gm
Day 2	1.039 gm	0.987 gm	0.954 gm
Day 3	0.972 gm	0.925 gm	0.862 gm
Day 4	0.916 gm	0.916 gm	0.703 gm
Day 5	0.900 gm	0.904 gm	0.701 gm
Day 6	0.761 gm	0.756 gm	0.625 gm

GRAPH II. GRAPHICAL REPRESENTATION OF TABLE IV.



F. Degradation rate of each microbe:

Formula = $\frac{W_A}{24}$

Degradation rate of SDB 1 = $\frac{0.12433333}{24}$ = **5.1 mg/hr**

Degradation rate of SDB 2 = $\frac{0.1088333}{24}$ = **4.5 mg/hr**

Degradation rate of SDB 1+SDB 2 combination = $\frac{0.054333}{24}$ = **2.2 mg/hr**

Degradation rate of SDF 1 = $\frac{0.0618}{24}$ = **2.5 mg/hr**

Degradation rate of SDF 2 = $\frac{0.079}{24}$ = **3.2 mg/hr**

Degradation rate of SDF 1+ SDF 2 combination = $\frac{0.0806}{24}$ = **3.3 mg/hr**

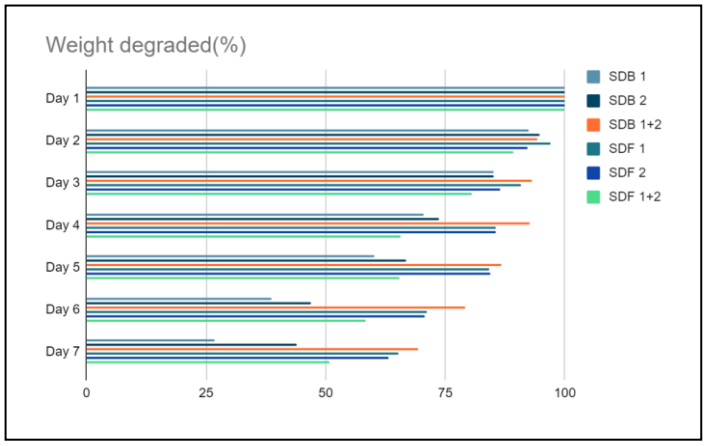
G. Percentage decrease in weight of bioplastic:

After 7 days, bioplastic in the SDB 1 broth had the most decrease in weight which was followed by SDB 2 and least in SDB 1 and 2 combination among the bacterial strains. Among fungi strains SDF 1 and 2 combination's broth had the most decrease in weight followed by SDF2 and least was shown by SDF 1.

TABLE V. DECREASE IN WEIGHT OF BIOPLASTIC AFTER EACH DAY

Days	SDB 1	SDB 2	SDB 1+2	SDF 1	SDF 2	SDF 1+2
Day 1	100%	100%	100%	100%	100%	100%
Day 2	92.5344%	94.7595%	94.3433%	97.1029%	92.243%	89.1589%
Day 3	85.0688%	85.2234%	93.0969%	90.8421%	86.4486%	80.5608%
Day 4	70.4323%	73.7114%	92.7134%	85.6075%	85.6075%	65.701%
Day 5	60.1179%	66.7526%	86.6731%	84.1122%	84.486%	65.5141%
Day 6	38.8016%	46.9042%	79.1947%	71.1215%	70.6543%	58.4113%
Day 7	26.7191	43.901%	69.3193%	65.3271%	63.2710%	50.8411%

GRAPH III. GRAPHICAL REPRESENTATION OF TABLE V.



As rate of degradation of each species was calculated, time required to degrade 1 gm of bioplastic was calculated as follow:

1. SDB 1: 8 days 4 hours
2. SDB 2: 9 days 6 hours
3. SDB 1 + SDB 2: 18 days 22 hours
4. SDF 1: 16 days 16 hours
5. SDF 2: 13 days 5 hours
6. SDF 1 + SDF 2: 12 days 15 hours

IV. CONCLUSION

Starch based bioplastics produced exhibit the same properties that of synthetic plastics i.e. being light weight, elastic, water resistant, shock resistant etc. Also provides some extra beneficiaries of being degradable and renewable so can be replaced in the place of synthetic plastics for greater good of the planet.

Bacterial and Fungal species found in soil have the ability to degrade starch based bioplastic since they can produce amylase enzymes that hydrolyses starch polymers into glucose monomers.

The degradation rate of the isolated bacteria and fungus were as follow :

1. SDB 1: 5.1 mg/hr
2. SDB 2: 4.5 mg/hr
3. SDB 1 + SDB 2: 2.2 mg/hr
4. SDF 1: 2.5mg/hr
5. SDF 2: 3.2 mg/hr
6. SDF 1 + SDF 2:3.3mg/hr

By studying the degradation rate of the isolated bacteria we can say that cocci shaped and rod shaped strains degrade at a higher rate compared to their combination which was lower than average. On the other hand the fungal species showed quite opposite results i.e. the combinations had higher rate of degradation rate compared to when their degradation rate was studied individually. When all the species of bacteria and fungi were to be compared then the highest degradation rate is shown by SDB 1 strain followed by SDB 2, SDF 2 and SDF 1 which has lowest degradation rate. Thus, it can be concluded that bacterial strains that were isolated have more degradation rate individually as compared to fungal strains degradation rate individually, but in combinations fungal species showed higher degradation rate as compared to bacterial species combination.

Time required for degradation of 1 gm of bioplastic by the isolated bacteria and fungus were as follow:
SDB 1: 8 days 4 hours, SDB 2: 9 days 6 hours, SDB 1 + SDB 2: 18 days 22 hours, SDF 1: 16 days 16 hours, SDF 2:13 days 5 hours, SDF 1 + SDF 2:12 days 15 hours.

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