



## EFFECT OF BIODEGRADED PLASTIC BLENDS ON THE GROWTH-RATE OF SOY BEAN PLANTS

Ifeoma P. Oragwu<sup>1</sup>

Department of Pure and Industrial Chemistry, Chukwuemeka Odumegwu Ojukwu University  
Anambra State, Uli, Nigeria. E-mail: [ifyporagwu@gmail.com](mailto:ifyporagwu@gmail.com) (+2348035721295)

**Abstract**— The effect of biodegraded low density polyethylene(LDPE) blends on the growth rate of soy bean plants was studied under the agricultural environmental conditions. Corn (*Zea Mayz*) starch which is a bio-filler was extracted, characterized, and incorporated into the synthetic low density polyethylene pellets, to initiate it's decomposition. The starch/plastic compatibility was improved by addition of a coupling agent(maleic anhydride-g-polyethylene), injection molded and extruded as sheets. Microbiological analysis of the soil sample was carried out to ascertain it's inhabitants. Some mechanical, water absorption, percentage weight-loss, and physical tests were carried out on the blended plastic blends for a period of 180 days before the plantation of the soya-bean seeds. The growth rate of the soya bean plants were monitored after germination at 7 days intervals for a period of 120 days. The growth rate of the plants was observed to increase, with the increase in starch contents, extent of decomposition and soil burial periods.

**Keywords:** Polyethylene blends, Soy-beans, Microbiological-tests, Biodegradation.

## INTRODUCTION

Biodegradation, simply means the integration, degradation, or assimilation of an organic substance by the action of microorganisms such as bacteria, fungi, enzymes or algae, and may result into loss of some chemical, physical or mechanical properties of the original substance.

This process can be initiated into synthetic or non-degradable polymer chains by the introduction of functional groups or additives, which can provide hydrophilicity, weak bonds, sites for microorganism or moisture attack on the polymer matrix, thereby degrading them (Amer, et al 2008). Degradable plastics are attracting a lot of attention due to the current interest in sustainability, recyclability, and environmental responsibility of widely used plastics like polyethylene (Friedrik et al 2002, Cheng, 2012).

Self-decomposing plastic-waste materials had been studied as a good source of organic manure for improving agricultural plant's yield. It can also help to control the release of dangerous gases like chlorofluorocarbon, carbon(11)oxide, which deplete ozone layer, resulting to global warming and green house effect due to incineration of those plastic wastes. (Bastioli, 2001). The non-degradability properties, outrageous utilization and indiscriminate disposal of plastic-materials, especially, the everyday single-used ones, like the bottles or sachet of water, milk, beverages, carriage bags, agricultural mulch, sanitary materials, disposable plates or cups, etc, had made plastics the controversial materials to the environmental waste management.

Starch is the major polysaccharide found in plant tubes and seeds endosperm which is biodegradable, cheap, regenerative, easily processable and non-toxic. Starch had been considered as suitable raw material for development of bio-plastics. Studies had shown that each granule of starch contains several millions of amylopectin (branched), with weaker bonded molecules, accompanied by amylose (linear) with stronger bonded molecules. Starch-blended polyethylene has a continuous phase that makes it hydrophilic and, therefore can catalyze enzymatic action. It had been reported that starch is the main source of carbon for microorganism growth (Ying Zheng et al (2005)).

Corn starch is chosen in this research due to its high amylopectin content ranging from 70 to 80% (Nadras et al, 2011). Addition of amylopectin molecules to the plastic films may adversely affect their flexibility, tensile properties, and cause their decomposition. Kang et al (1998) and Watanabe et al (2009) had reported that, when starch granules

were embedded into polyolefin matrix, the polysaccharide-loving microorganisms, enzymes, fungi, etc, were attracted to feed on them and consequently initiate their degradation. They observed that gradual removal of the starch debris alternatively created voids and pits on the blend's surface, resulting into penetration of moisture and weakening of the interfacial force of attraction (Yamad-Onodera et al, 2001).

Breslin (1993) had proved that pure(neat) plastic sheets remained unchanged after two years of soil burial when subjected to the same biodegradation conditions with starch/plastic blends. Hydroxyl groups in the starch molecule could be introduced into the matrix of plastics to make them hydrophilic, initiate their biodegradation in-order to make them eco-friendly (Shrogen et al, 2003).

Complete mineralization of the blend samples can occur when biodeterioration, biofragmentation, and assimilation could be achieved in an abiotic or biotic conditions.

Starch –based plastics had been proved to have no negative –health effects on quality of food or other packaged materials. The health standard of the populace will improved by eradicating plastic wastes, which are the commonest breeding sites for some disease causing insects, like mosquitoes, cockroaches, flies, etc.

However, development of biodegradable plastics had been thought of as the easiest way to ensure a cleaner, greener, healthier, eco-friendly environment, improvement to crop yields, and stability in our food chains. As part of our contribution to minimize plastic waste menace, corn starch was incorporated into non-biodegradable low density polyethylene matrix to initiate it's biodegradation within a specific period of time. This waste was reconverted into manure to which can be used to enrich our farm land in order to improve agricultural yield.

## **2.0 Materials and Methods**

### **2.1 Materials**

#### **a. Equipment/ apparatus**

The injection molding machine and the steel mould used in this research were gotten from CEEPLAST Industry, Aba, Abia State. Instron Tensile Strength Machine was gotten from CUTIS Industries Nnewi, Anambra State. The Austell Scientific Auto-clave, Microscope, Glass slides, Specimen bottles, Plastic buckets, Dessicator, Refrigerator, Digital weighing balance, and pH meter were gotten from Chemistry and Microbiology Laboratories of Anambra State University, Uli, Anambra, State, Nigeria.

#### **b. Chemicals/Materials**

Humus soil samples used for the biodegradation tests were gotten from the composite site at Anambra State University Uli. The compatibilizer (Malei anhydride-graft-polyethylene) and Polyethylene pellets were purchased from FINLab Chemical Stores, Owerri, Imo State. Corn starch was extracted from *zea mayz*, while soya bean seeds were gotten from College of Agriculture Mgbakwu, Anambra State.

### **2.2 Sample Preparation**

#### **Characterization of Corn Starch**

Steeping process was used to extract the corn starch according to the patent US 801226282(2011)specification. Purification, characterization and other tests, like percentage moisture content, particle size, ash content, and iodine test, etc, were carried out on the extracted corn starch sample to ensure it's suitability in blending with low density polyethylene.

### **2.3 Extrusion of the Plastic/Corn Starch Blends**

Known weight of the plastic pellets and corn starch were mixed and extruded as sheets using injection molding machine at the operation temperature of 250°C. Another set of compatibilized blends were prepared by incorporating a coupling agent (Maleic anhydride graft Polyethylene) at 0 to 6 wt. % by mass of the starch content. Pure Polyethylene sheets which were to be used as control samples were also extruded under the same temperature and pressure conditions.

### **2.4 Mechanical Properties of Low density polyethylene /Corn Starch Blends Before Microbial Tests**

#### **(i) Tensile Strength (TS)**

The blended sheets were cut into dumb-bell shapes and subjected to tensile testing, by clamping them to the jaw of the Instron Tensile Testing Machine (TTM2EL/0300/2005). The breaking force were recorded and the

tensile properties calculated according to ASTM D 638 standard. The effect of starch granules to the polyethylene matrix was investigated by subjecting the pure polyethylene sheets to the same test and comparing their values.

## (ii) Elongation at Break(%)

The elongation at break (EB) of the blended samples were recorded along the meter rule pointer on the tensile testing machine and the average values calculated. The properties of pure plastic films to be used as control samples were also determined before and after biodegradation tests.

## 2.5 Soil Burial Test

26 plastic buckets of volume 2000 cm<sup>3</sup> were perforated underneath, filled with 1 kilogram of humus soil, and moist with water. Weighed samples of polyethylene/corn starch blend films were buried at a depth of 10 cm from the soil surface for a period of 200 days. The control samples(pure low density polyethylene) were also buried in the soil under the same conditions. The extend of biodegradation of the samples was determined at regular time intervals (30 days) by removing the samples from the soil, washed gently with distilled water, and dried in an air oven at 40 °C to a constant weight. The weight- loss was determined every 30 days from the starting days, and was calculated using:

$$\text{Weight loss (\%)} = \frac{w_i - w_f}{w_i} \times \frac{100}{1} \quad \text{1.0}$$

where:  $w_f$  is the weight of the film after soil burial

$w_i$  is the initial dry weight of the film.

## 2.6 Microbiological Determination of the Soil Samples

The microbial inhabitants of the soil samples were determined after sterilizing the materials like conical flasks, test tubes, petri-dishes, metal rods, glass slides, iron loop, etc, by washing with clean water, and detergent. These materials were kept in Astell Scientific auto-clave at a high pressure and saturated steam of 121 °C for 20 minutes before use.

### (a) Preparation of Normal Saline

The normal saline was prepared by dissolving 0.9 g of sodium chloride in 100 cm<sup>3</sup> of distilled water, and transferred into different test-tubes. The soil sample homogenate was prepared by transferring 1 cm<sup>3</sup> of the soil mixture into each of the test-tubes containing 10 ml of the normal saline, and labeled as 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup>. 1 cm<sup>3</sup> of the soil

homogenate was pipetted from the test tubes labeled  $10^{-1}$  into  $10^{-2}$ , while  $10^{-2}$  was transferred into  $10^{-3}$ , till  $10^{-6}$  in order to distribute the soil microorganisms.

### **(b) Identification of Microorganisms**

The soil samples and known mass of nutrient agar (Master Biology Brand) homogenate was prepared using spread plate technique, incubated for 48 hours at the temperature of  $37^{\circ}\text{C}$  to allow for the growth of the microorganisms. Different colonies were picked and sub-cultured on the agar plates containing  $15\text{ cm}^3$  of the media and allowed for another 24 hrs incubation period.

### **(c) Isolation and Biochemical Identification the Soil Microbes**

Different organisms present in the soil samples were identified in the culture by their gram positive or negative reactions. The different colonies of the organisms were collected and smeared on slides each containing a drop of distilled water and examined using a microscope to ensure the distribution of the bacteria cells, spore staining, motility test, etc. Stock cultures of various isolates were used to confirm the behavior of individual organism by carrying out some tests like coagulase, citrate, catalase, oxidase, etc.

## **2.7 Effect of biodegraded products of low density polyethylene on the growth-rate of soya bean plants**

The effect of biodegradation products of low density polyethylene/corn starch blends on the growth-rate of soya bean plants was determined by monitoring the growth of soybean plants stem from germination stage according to ([www.soybeanstation.org](http://www.soybeanstation.org)) specifications. The length of the stem and shoot of the growing plants were measured, and compared to that of the pot containing pure polyethylene films for 42 days at 7 days intervals.

### **3.0 RESULTS AND DISCUSSION**

#### **3.1 Tensile Properties of Polyethylene/Corn Starch Blends Before Soil Burial**

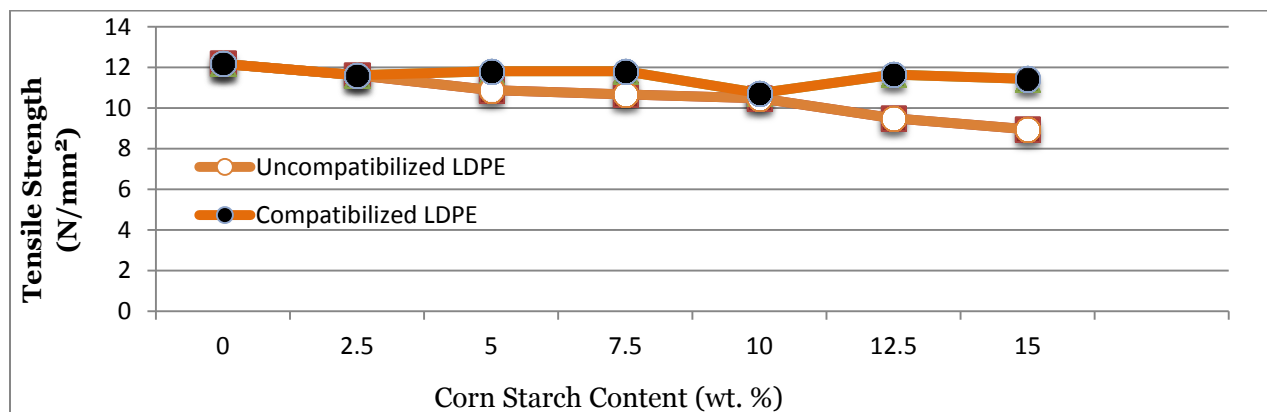
##### **(a) Tensile Strength(TS) and Elongation at Break(EB)**

The mechanical properties (tensile strength and elongation at break) of the LDPE/Corn Starch blends are illustrated in Table 1 /Figure 1 and Table 2/ Figure 2 respectively. These properties were observed to decrease from 11.61 to 8.95 N/mm<sup>2</sup>, for TS and from 206.65 to 29.30 % for EB at starch content of 2.50wt.% to 15.00wt.% respectively, for uncompatibilized blends. The pure polyethylene samples showed highest tensile strength value of 12.17 N/mm<sup>2</sup> and elongation at break of 444.65 % through out. The drastic reduction in values of these properties as compared to the pure polyethylene films, could be attributed to the incompatibility in the bonding strength of plastics and the biopolymers. It could also be attributed to the heterogeneous dispersion of starch granules within the polyethylene matrix. Starch molecules cannot stretch-out, therefore, cracks can occur to reduce the elongation at break of the polyolefin/starch blends. Our observations were in conformity to the findings of Martins, et al(2001); Obasi(2013) and Kang, et al(1998), who reported that slight increase in starch content decreased the elongation at break and tensile strength of sweet potato/ starch blended polypropylene. Asha and Richard (1994), and Moris et al (2001) who carried out biodegradation tests on some plastics also reported decrease in the tensile strength of plastics with increased corn starch content, and extension of period of soil burial.

These mechanical properties were significantly improved with the incorporation of maleic anhydride graft polyethylene. It was assumed that the hydroxyl(OH) functional groups from starch, and that of MA-g-PE were the source of interfacial interaction between the films (Nopparut and Kawee, 2013). The improved interfacial adhesion thus played an important role in the process of stress transfer, thus reducing the chance of interfacial debonding, and loading to increased tensile strength, and elongation at break as observed in the compatibilized blends.

**Table 1: Values for Tensile Properties of Pure and Corn-starch Blended Polyethylene Before Biodegradation Tests**

Starch content (wt.%)	LDPE content (wt.%)	Tensile Strength (N/mm <sup>2</sup> )	
		Uncompatibilize LDPE	Compatibilized LDPE
0.00	100.00	12.17	12.17
2.50	97.50	11.61	11.61
5.00	95.00	10.88	11.81
7.50	92.50	10.67	11.81
10.00	90.00	10.48	10.73
12.50	87.50	9.49	11.64
15.00	85.00	8.95	11.43

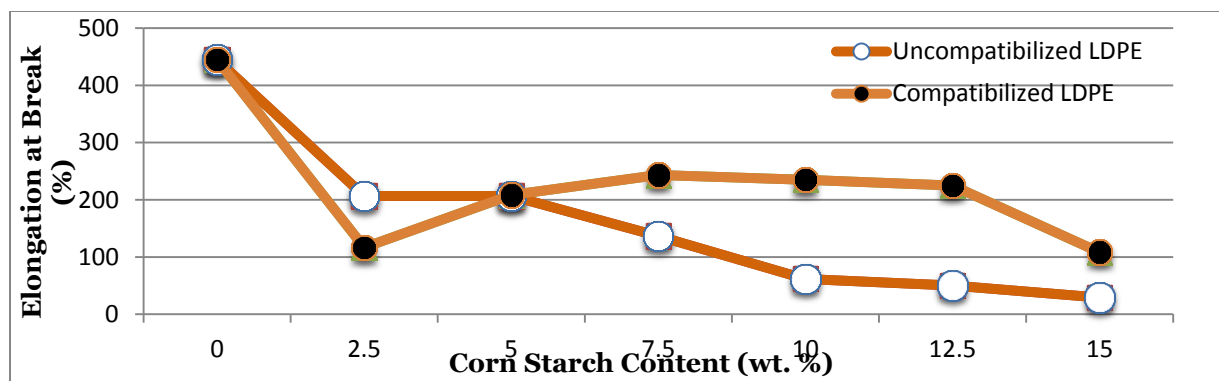


**Figure 1: Plot of Tensile Strength of Compatibilized and Uncompatibilized LDPE /Corn Starch Blends.**

**Table 2: Elongation at Break(%) of Uncompatibilized and Compatibilized Low Density Polyethylene Blends before Biodegradation Test.**

Corn Starch (wt.%)	LDPE (wt.%)	Elongation at Break (%)	
		Uncompatibilized LDPE	Compatibilized LDPE
0.00	100.00	444.65	444.65
2.50	97.50	206.65	116.67
5.00	95.00	206.65	208.33
7.50	92.50	136.65	243.33
10.00	90.00	61.65	235.00
12.50	87.50	50.20	225.00
15.00	85.00	29.30	108.33





**Figure 2: Plots of Elongation at Break(%) of Uncompatibilized and Compatibilized Low Density Polyethylene Blends before Biodegradation Test.**

**Table 3: Results on the Biochemical Characteristics of Different Isolates from the Soil Sample.**

Characteristics	St	E-coli	Sa	Ba	Ps	La
Gram reaction	+	—	—	+	+	—
Shape	Spherical	Rod	Coma	Rod	Rod	Rod
Size (mm)	1.0	2.2	1.4	1.8	2.5	4.2
Spore test	—	+	—	—	+	—
Motility	—	+	—	—	+	+
Indole test	+	—	—	—	—	—
H <sub>2</sub> S	—	—	—	—	—	+
Coagulase	+	—	—	—	—	—
Citralase	—	—	—	+	+	—
Catalase	+	+	+	+	+	—
Oxidase	—	—	—	—	—	+
Appearance	Yellow	White	White spread	Bluish grey	Bluish grey	Bluish

**Keys:** St— *Staphylococcus aureus*; Ps— *Pseudomas sp.*; E- coli,  
La— *Lactobacillus sp.*; Ba— *Bacillus sp.*; Sa— *Samonella sp.*

The dominant microbial species(bacteria) identified in the soil sample used for the soil burial tests were *Staphylococcus aureus*, *E. Coli*, *Samonella Sp.*, *Bacillus Sp.*, *Pseudomanas Sp.*, and *Lactobacillus Sp.*, as shown on table 3 above. *Pseudomanas Sp.* and *Staphylococcus aures* were observed to contribute to the decomposition of the polyethylene blends according to the findings of Kathiresan(2003) who studied the degradation of polyethylene blends buried in mangrove soil and observed that the major microorganisms involved in the degradation were *Pseudomanas Sp.* and *Staphylococcus aures*. It was observed that as the starch content increased,

the polymer blends became more susceptible to biodegradation, because the agents of degradation seems to multiply and their actions also increased. This could also be attributed to the increase in the surface area of blended plastic films which exposed them to more microbial attack. Pure polyethylene remained the same throughout the biodegradation period because of its stability, inertness, structure of the matrix and long chains of the ethylene monomers that cannot be easily degraded by microorganisms. This is in conformation with the reports of Kaur and Ecautam, 2010; Behjat et al, 2009), that these polyolefins can stay for years and are seen littering our environment because they are not degradable by any agent of degradation.

### 3.3 Percentage Weight-loss

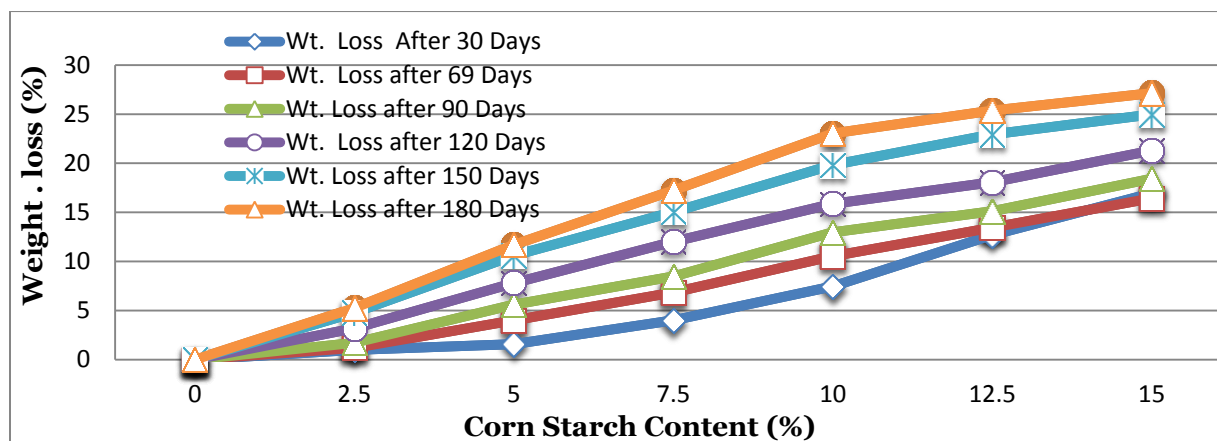
There was no apparent weight-loss for the pure LDPE throughout the entire soil burial periods, showing that pure polyethylene is inert to uv- radiation, air, moisture and microbial attack. The plots show continuous increase in the weight-loss of LDPE/corn starch blended sheets with increased starch contents. It was observed that the percentage weight-loss increased between 30 to 180 days, from 0.99 wt.% to 27.14 wt. % at starch content from 2.50 wt.% to 15.00 wt.% as shown on Table 4/Figure 4. The reduction in the weight of the blends samples could be due to the removal of starch granules by soil microorganisms. It is expected that extension of the biodegradation period could result into complete bio-fragmentation, assimilation and mineralisation of the blended polyethylene. Albertsson (1998) had reported that the penetration of small amount of solar radiation reaching the film under soil surface might initiate oxidation process leading to complete degradation of the polymer blends. This process may result into conversion of the monomers to carbon (1v)oxide, nitrogen, methane, water, biomass, which can combine with the minerals compounds in the soil to improve their nutrients, that are needed for plant growth (Ghosh et al(2013).

The compatibilized LDPE blends showed reduced weight loss when compared to the uncompatibilized blends throughout the soil burial period due to the increased adhesion, and bonding effect of MA-g-PE to the polyolefin matrix and corn starch granules as shown on Table 5/ Figure 5. The weight-loss for compatibilized LDPE, blended with 2.5 wt. %, and 5.0 wt.% corn starch after 30 to 90 days of soil burial tests were low when compared to the uncompatibilized blends. This also could be attributed to

(i) the short time for biodegradation, and (ii), low content of corn starch to render biodegradation.

**Table 4: Weight Loss for Uncompatibilized Low Density Polyethylene / Corn Starch Blends**

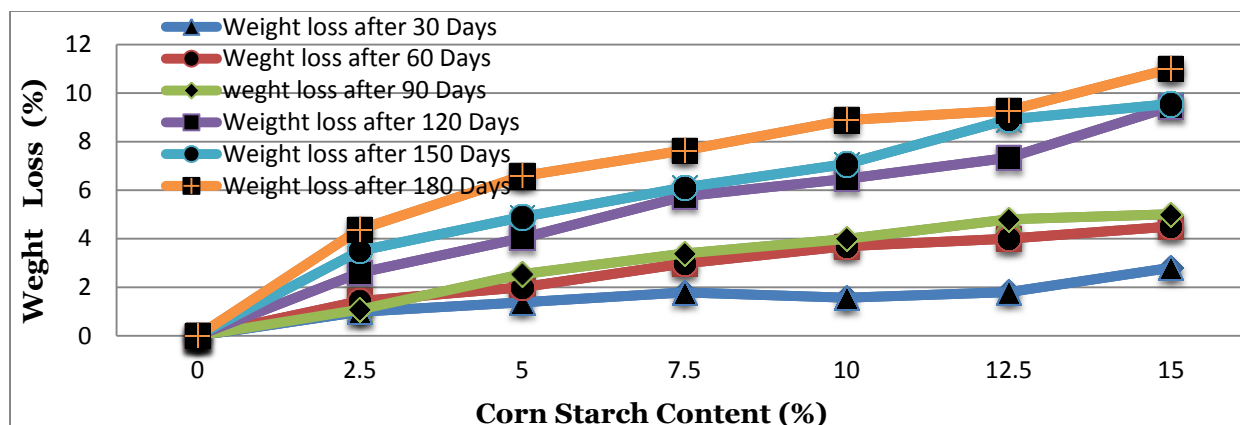
Days	Weight Loss (wt.%)						
	LDPE	Corn Starch Content (%)					
		2.50	5.00	7.50	10.00	12.50	15.00
<b>30</b>	0.0	0.99	1.58	4.00	7.75	12.68	16.94
<b>60</b>	0.0	1.19	4.00	6.84	10.53	13.43	16.43
<b>90</b>	0.0	1.71	5.60	8.46	12.90	15.11	18.43
<b>120</b>	0.0	3.18	7.80	12.00	15.86	18.06	21.29
<b>150</b>	0.0	4.78	10.60	15.0	19.82	22.94	24.97
<b>180</b>	0.0	5.25	11.70	17.24	23.04	25.37	27.14



**Figure 4: Plot of Weight loss of Uncompatibilized Low Density Polyethylene / corn Starch Blends**

**Table 5: Weight Loss for Compatibilized Low Density Polyethylene/ Corn Starch Blends.**

Days	Weight Loss (%)						
	LDPE	Corn Starch Content (wt. %)					
		2.50	5.00	7.50	10.00	12.50	15.00
<b>30</b>	0.0	0.99	1.38	1.79	1.57	1.80	2.80
<b>60</b>	0.0	1.43	2.00	2.98	3.69	4.00	4.50
<b>90</b>	0.0	1.08	2.57	3.39	3.99	4.80	5.01
<b>120</b>	0.0	2.60	4.03	5.75	6.47	7.34	9.44
<b>150</b>	0.0	3.50	4.89	6.11	7.08	8.91	9.55
<b>180</b>	0.0	4.39	6.58	7.64	8.89	9.28	11.00

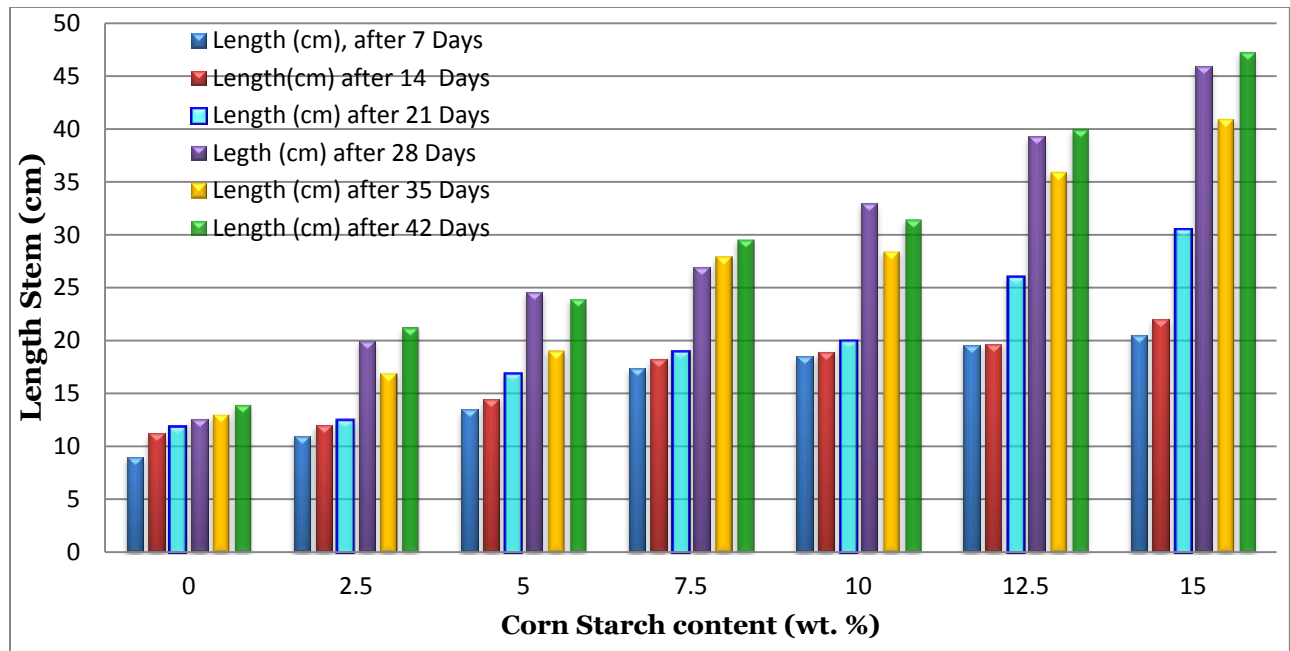


**Figure 5: Plot of Weight loss of Compatibilized Low Density Polyethylene and Corn Starch Blends.**

#### 4.5 Effect of Products of Biodegraded Polyolefin Blends on the Growth of Soya Bean Plants

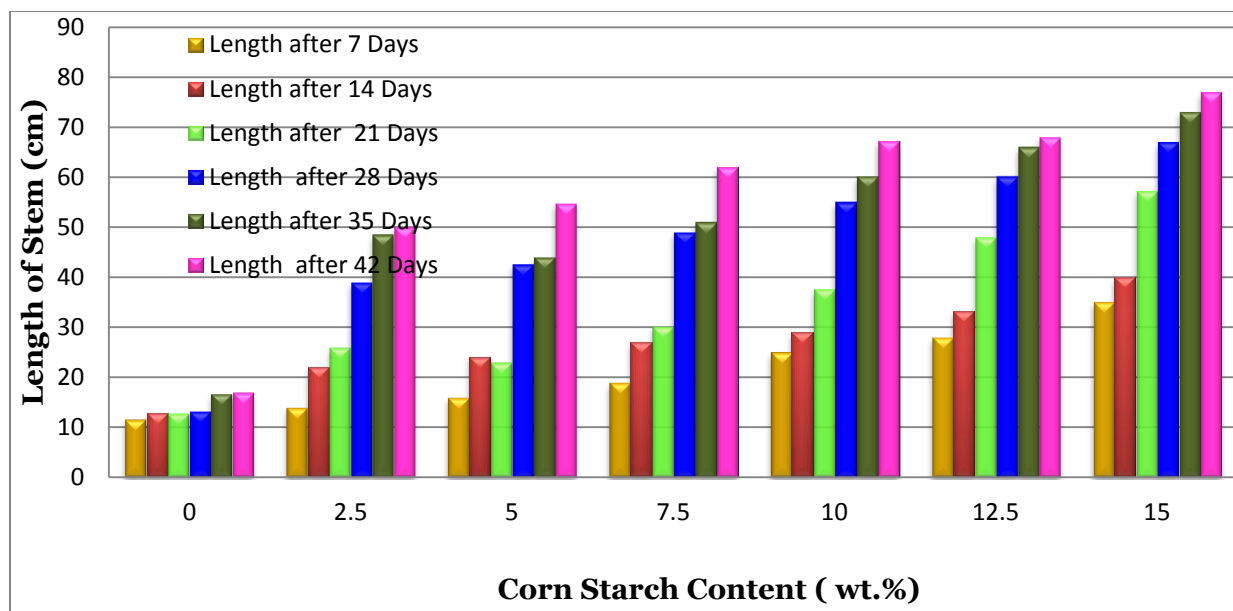
The results of the measurement obtained on the growth of soya bean plants are illustrated in Figures 6 and 7. The charts showed retarded length of 9 to 14 cm<sup>3</sup> after 7 days for soya bean plants grown in pure polyethylene samples which were used as control samples. There were gradual growth in the length of the stems of soya bean plants with increased corn starch contents throughout the growth periods. The growth-rate for the stems of soya bean planted in soil containing compatibilized polyethylene blends as illustrated on Figure 6 were less than those planted in soil containing uncompatibilized polyethylene blends as shown in figure 7. This shows that uncompatibilized polyethylene blends degraded the more, and mineralized to improved the soil fertility, which the soya bean plants utilized for their nutrient and growth. It is worthy to note that soil containing biodegraded polyethylene blends were observed to be darker in colour than the ones containing the pure samples. It is assumed that there is increase in the absorption of solar energy that introduced nitrogenous compounds which has the ability to improving the soil fertility (<http://soils.usda.gov/sqi> (2008)).

Thus, the widespread application, utilization of biodegraded plastics in sustainable farming can result into higher yield of food products (Petkewich, 2003).



**Figure 6: Growth Rate of Soya Bean Plant in Soil containing Biodegraded Compatibilized Low Density Polyethylene**

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**Figure 7: Growth Rate of Soya Bean Plant in Soil containing Biodegraded Uncompatibilized Low Density Polyethylene**

## Conclusion

Low cost, efficient technology, eco-friendly treatments capable of reducing and even eliminating plastics, are of great environmental interest. Among the biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of plastics and converting their products to green plants nutrient. There is a huge demand in exploring these microbes which can grow in different soil conditions or specific stress conditions, to use the plastic carbon atoms as energy source to enrich farm land nutrients. Our reports had shown that starch blended plastic materials can degrade or fragment, mineralized naturally to form carbon dioxide, water, inorganic compounds, biomass, etc, that can serve as fertilizers in conditioning agricultural farm lands.

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