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Degradation behavior of biocomposites based on cassava starch buried under indoor soil conditions

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ABSTRACT

Degradation of cassava (tapioca) starch based composite films during indoor soil burial experiments was analyzed using five factors, three levels Box–Behnken response surface design. From the results, it was observed that, increased water sorption promotes the entry of soil microorganism and it utilizes the starch films as a source of energy for their growth. The reduction in weight and mechanical property was associated with preferential loss of matrix components of the films. The microorganisms associated with the degradation of films were quantified and identified. Scanning electron microscopy (SEM) analysis showed the formation of patterns and cracks on the surface of the materials aged in the soils. From the results, second order polynomial models were developed for the responses. The results of the study demonstrated that, the tapioca starch based composites were showed a limited lifetime in biotic environment which make them suitable for being disposed in landfills after their use.

1. Introduction

Growing consumption of polymeric materials caused the increase in solid waste production. Plastic wastes accumulating in the environment are posing an ever increasing ecological threat. When the solid plastic waste is present in the soil, reduces the soil fertility and prevents the growth of plant life and possess the environmental problems. Hence, development and characterization of environmentally friendly polymeric materials have attracted extensive interest owing to the environmental problem induced by the accumulation of plastic waste (Siracusa, Rocculi, Romani, & Rossa, 2008; Weber, Haugaard, Festersen, & Bertelsen, 2002). In recent years biopolymers have been seen as potential environmentally friendly and sustainable alternatives to petroleum based plastics, particularly in those applications where biodegradability and derivatization of natural resources give added value (Avella et al., 2005).

Development of edible and biodegradable films using natural biopolymers such as starches could also be useful in replacing synthetic non-biodegradable packaging in some applications (Chen, 1995) and have received remarkable attention globally as they are totally eco-friendly and helpful in waste landfill management. The use of starch as a material for preparation of edible or biodegradable films has been widely recognized (Krochta & De Mulder-Johnston, 1997), starch possesses many unique properties and considered as one of the most promising natural renewable resources because of its lower cost, biodegradability, thermoplastic behavior (Mali et al., 2005) and availability in abundance than other natural resources. Starch has been applied in the field of degradable plastics and blend films containing starch are potential packaging materials in the agriculture, medicine, and packaging industries (Funke, Bergthaller, & Lindhauer, 1998; Hulleman, Janssen, & Feil, 1998; Lu, Tighzerta, Dole, & Erre, 2005). Besides, upon disposal, they are completely degraded by micro-organisms in various environments such as soil, sea lakes and sewage (Thiré, Ribeiro, & Andrade, 2006), did not have a negative effect on the environment and also reduced the green house effect (Bastioli, 2001). Surfactants could be incorporated in the film formulation to reduce the surface tension of the solution by improving wettability and adhesion of the films.

Studies on starch based biodegradable films with respect to its biodegradation behavior are important for their application in environment. Biodegradation is a biochemical transformation of compounds by microorganisms and the propensity of a material to get breakdown into its constituent molecules by natural processes. Among various methods of degradation, soil burial method is one of the frequently used methods for the determination of biodegradability of polymer films (Yang, Yoon, & Kim, 2005). Microorganisms (bacteria and fungi) present in the soil could influence the degradation of film by using it as their food source in the natural environment. Hence, the present study is focused

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on the biodegradation of eco-friendly biocomposites based on tapioca starch was exposed to natural mixed micro flora present in soil during indoor burial conditions and it can be considered as a realistic approach to the biodegradation process in the natural environments. Water sorption (WS %) and weight loss (WL %) during soil burial were evaluated gravimetrically. The effect of biodegradation on the mechanical properties of the films was also analyzed. Morphological characterization of the biodegraded specimens was carried out by scanning electron microscopy (SEM). In addition, microorganisms responsible for degradation such as bacteria and fungi were isolated and enumerated. The degradation behavior was quantitatively analyzed in order to develop suitable mathematical models for describing the effects of process variables such as tapioca starch, glycerol, agar, span80 and time on the soil burial degradation properties of tapioca starch based films using five factors three levels Box–Behnken response surface design (BBD). Because, BBD is a spherical, revolving design and also it does not contain combinations for which all factors are simultaneously at their highest or lowest levels. So this design is useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results might occur (Ferreira et al., 2007).

2. Materials and methods

2.1. Raw materials

Tapioca starch was obtained from local market, Erode, Tamil Nadu, India. Amylose, amylopectin, ash, moisture and starch content of tapioca starch were determined and reported elsewhere (Prakash Maran, Sivakumar, Sridhar, & Prince Immanuel, 2013). Glycerol (98% purity) and span 80 (Sorbitan monooleate, 99% purity) were purchased from Merk chemicals, Mumbai, whereas Agar was purchased from Hi-media chemicals, Mumbai. Soil was taken from the campus of Kongu engineering college, Perundurai, Erode, from the surface layer of the ground. All inert materials were carefully removed to obtain a relatively homogeneous mass.

2.2. Preparation of films

The film forming solutions were prepared from various proportions of starch (1–3 g), glycerol (0.5–1.0 ml), agar (0.5–1.0 g) and span-80 (0.1–0.5 ml). Casting method was employed to prepare the films and the details are given in elsewhere (Prakash Maran et al., 2013). The prepared homogenous and transparent films were separated from Petri plates and equilibrated at 25 °C, 58% relative humidity for 72 h prior to the experimental analysis.

2.3. Indoor soil burial degradation

Soil burial degradation tests were performed according to the method described by Di Franco, Cyras, Busalmen, Ruseckaite, and Vazquez (2004) in a series of plastic boxes (80 cm × 15 cm × 10 cm) containing soils and the natural microflora present in the soil was used as the degradation medium for films. The pH of the soil was 6.7. Each film sample were cut into rectangular shape ($2 \times 10\,cm^3$) and dried in an oven until it attains the constant weight. Then the dried film samples were buried at the depth of 8 cm from the surface of the soil. The plastic boxes containing samples were incubated at room temperature ((28–35 °C) and humidity of the soil was maintained at 20–40% by sprinkling of water at regular interval through out the study (30 days). The degradation of the specimen was determined at a regular time interval (5 days) by taking the specimen carefully from the soil and washing it gently with distilled water to remove the soil. Then the sample was dried under vacuum until a constant was obtained. Weight loss (WL) during soil burial was measured according to Rafiemanzelat, Zonouz, and Emtiazi (2012). The mass of each sample was weighed before and after degradation and weight loss of each film sample was obtained using the following formula:

$$WL(\%) = \frac{M_0 - M_1}{M_0} \times 100 \quad (1)$$

where M_0 is the pre-degraded dry weight of the polymer and M_1 is the dry weight of the sample after degradation.

Water sorption (WS) was determined according to method described by Martucci and Ruseckaite (2009). After a specified period of time (t), films were removed from the soil, thoroughly rinsed with tap water, and dried with a tissue paper to remove excess water from their surfaces and weighed (W_h). Water absorption was calculated by the following equation:

$$WS(\%) = \frac{W_h - W_t}{W_0} \times 100 \quad (2)$$

where W_0 is the initial mass, W_t is the remaining mass due to biodegradation at time (t) and W_h is the humid mass.

Materials deterioration and weight loss were accompanied by loss in their mechanical properties. Mechanical properties of the films (tensile strength and elongation) were measured using a material testing machine (Universal tensile strength tester) with 3 kg load cell. The mechanical properties of the films before soil burial test were reported elsewhere (Prakash Maran et al., 2013). The losses in the mechanical properties were calculated using the following equation (Alvarez, Ruseckaite, & Vazquez, 2006)

$$\frac{P_i - P_f}{P_i} \times 100 \quad (3)$$

where P_i is the selected measured mechanical property of the material before burial test and P_f is the selected measured mechanical property of the material after burial test

2.4. Microbial count

Microorganisms (bacteria and fungi) present in the degraded films were analyzed by the procedure suggested by Vijaya and Reddy (2008). The sterile forceps were used to collect the film samples from the soil. The samples are washed gently with sterile distilled water to remove soil debris present in the films. About 1 g of the sample infested with microorganisms was transferred in to a conical flask containing 99 ml of sterile distilled water. The content was shaken vigorously and serially diluted. Pour plate method was adopted to isolate the microorganisms associated with the material. Zobell's agar medium and Martin Rose Bengal agar medium was used to isolate the bacteria and fungi present in the material. For each dilution (10^6 for bacteria and 10^3 for fungi), three replicates were made and the plates were incubated at 30 °C for 2–7 days. The colonies present in the plates were counted (bacterial count (BC) and fungal count (FC)) with the help of a digital colony counter (Toshiba, India) and are referred as colony forming unit (CFU) per gram of sample.

2.5. Identification of microorganisms

Among the bacterial and fungal colonies observed in the plates, the dominant colonies were isolated and sub-cultured repeatedly for getting pure cultures and are preserved in slants for further identification. The bacterial strains which are present in the preserved slant cultures were identified by the method suggested by Oliver (1982). For Gram-negative bacteria, motility, glucose oxidation, penicillin sensitivity and glucose fermentation tests were conducted. Shape, dextrose fermentation, catalase and glucose fermentation tests were conducted for Gram-positive bacteria. The

Table 1
Box–Behnken design matrix and observed results.

Run order	Starch (g)	Glycerol (ml)	Agar (g)	Span80 (ml)	Time (days)	WS (%)	WL (%)	TS (%)	E (%)	BC (cfu/g)	FC (cfu/g)
1	2	0.5	0.5	0.3	15	35.94	46.84	43.28	55.87	38	21
2	1	0.5	0.75	0.3	15	35.24	48.46	44.28	53.21	36	20
3	2	0.75	0.75	0.1	30	33.27	45.32	41.86	55.47	37	20
4	2	0.75	0.75	0.3	15	37.23	53.42	50.29	61.21	43	23
5	3	0.75	0.75	0.5	15	36.24	48.57	44.98	56.28	39	21
6	3	0.75	0.75	0.1	15	35.84	46.54	42.58	56.84	36	21
7	2	0.75	1	0.1	15	33.54	45.84	41.26	52.34	35	19
8	1	1	0.75	0.3	15	37.02	52.14	49.35	58.24	36	21
9	1	0.75	0.75	0.5	15	31.68	43.25	39.54	52.84	34	21
10	2	0.75	0.5	0.5	15	34.18	43.29	40.01	51.34	35	20
11	2	0.5	0.75	0.3	0	0	0	0	0	0	0
12	2	1	0.75	0.5	15	35.84	45.83	42.51	54.34	36	21
13	2	0.75	0.75	0.3	15	37.23	53.42	50.29	61.21	43	23
14	2	0.75	0.75	0.3	15	37.23	53.42	50.29	61.21	43	23
15	2	0.75	0.75	0.3	15	37.23	49.63	47.58	54.28	39	21
16	2	0.5	1	0.3	15	33.54	45.24	43.19	57.28	36	20
17	1	0.75	0.5	0.3	15	35.89	50.84	48.58	57.24	37	20
18	2	0.75	0.5	0.3	30	36.54	49.66	46.58	55.28	37	21
19	2	1	0.5	0.3	15	35.84	46.27	43.58	55.27	37	21
20	2	1	0.75	0.3	0	0	0	0	0	0	0
21	1	0.75	0.75	0.3	30	34.58	45.21	42.58	54.68	35	20
22	2	0.75	0.75	0.3	15	37.23	53.42	50.29	61.21	39	21
23	2	1	0.75	0.3	30	34.84	46.02	42.58	53.08	35	21
24	1	0.75	0.75	0.1	15	32.98	40.48	36.48	41.85	33	19
25	2	0.75	0.75	0.5	30	31.84	42.54	38.54	49.35	33	21
26	2	1	1	0.3	15	34.28	45.28	42.06	54.28	35	20
27	2	0.75	0.75	0.3	15	37.28	46.52	43.36	55.28	39	22
28	3	0.5	0.75	0.3	15	35.98	44.25	41.58	52.48	36	21
29	3	0.75	0.75	0.3	0	0	0	0	0	0	0
30	2	0.75	1	0.5	15	34.58	45.62	41.58	54	35	20
31	3	0.75	0.75	0.3	30	29.54	38.54	36.54	42.98	32	18
32	2	1	0.75	0.1	15	32.54	41.58	38.45	48.39	32	20
33	3	1	0.75	0.3	15	32.58	42.85	38.28	48.24	34	21
34	1	0.75	0.75	0.3	0	0	0	0	0	0	0
35	2	0.5	0.75	0.3	30	34.28	46.84	42.58	55.37	37	21
36	2	0.75	1	0.3	0	0	0	0	0	0	0
37	3	0.75	1	0.3	15	34.98	47.24	44.38	57.68	37	20
38	2	0.75	0.75	0.1	0	0	0	0	0	0	0
39	2	0.75	0.5	0.1	15	33.54	42.54	39.25	49.28	34	20
40	3	0.75	0.5	0.3	15	34.54	47.64	43.58	54.32	32	19
41	2	0.5	0.75	0.5	15	34.27	45.32	41.22	52.83	35	20
42	2	0.75	0.75	0.5	0	0	0	0	0	0	0
43	2	0.75	1	0.3	30	34.08	43.54	42.15	56.38	35	20
44	2	0.75	0.5	0.3	0	0	0	0	0	0	0
45	1	0.75	1	0.3	15	31.98	45.07	37.65	50.63	30	18
46	2	0.5	0.75	0.1	15	33.68	44.28	41.86	53.01	34	20

fungal strains were identified after staining them with cotton blue stain (Vijaya & Reddy, 2008).

2.6. Morphology analysis

To examine the surface morphology of the film (before and after degradation), scanning electron microscope (SEM) (FEI Quanta FEG-200, USA) was used. Samples were coated with a thin layer of gold to avoid sample charging during imaging and then examined under SEM. The microstructure of the degraded films were magnified and digitally recorded.

2.7. Experimental design and statistical analysis

A Box–Behnken response surface experimental design (BBD) with five factors (starch (X_1), glycerol (X_2), agar (X_3), span-80 (X_4) and burial time (X_5)) at three levels (Table 1) was performed to study and investigate the individual and interactive effects of process variables on the soil burial degradation properties such as water sorption (WS %), weight loss (WL %), tensile strength (TS, %), elongation (E, %), bacterial count (BC, cfu/g) and fungal count (FC, cfu/g) of tapioca starch based films. For each factor, an experimental range was selected based preliminary single-factor experimental results. The range of independent variables and their levels are presented in Table 1 and it was coded (−1, 0 and +1) according to the method described by Maran, Sivakumar, Sridhar, and Thirugnanasambandham (2013) for statistical calculation. Forty six experiments (including six center points) were conducted in a randomized order and the data were analyzed by multiple regression analysis in order to develop an empirical second order regression polynomial mathematical model for the responses, which exhibits the relationship between response and independent variables. The generalized form of second order polynomial mathematical model was given in elsewhere (Prakash Maran, Mekala, & Manikandan, 2013). The construction and analysis of the experimental design, Pareto analysis of variance (ANOVA) and quality of fit of the polynomial model (coefficient of determination (R^2), adjusted coefficient of determination (adj-R^2) and predicted coefficient of determination (pre-R^2)) was analyzed with the help of a statistical software package (Design-Expert 8.0.7.1, State-Ease Inc., Minneapolis, MN, USA). The regression coefficients of the developed polynomial models were used to construct the three dimensional (3D) response surface plots in order to visualize the relationship between independent variables and the responses (Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013).

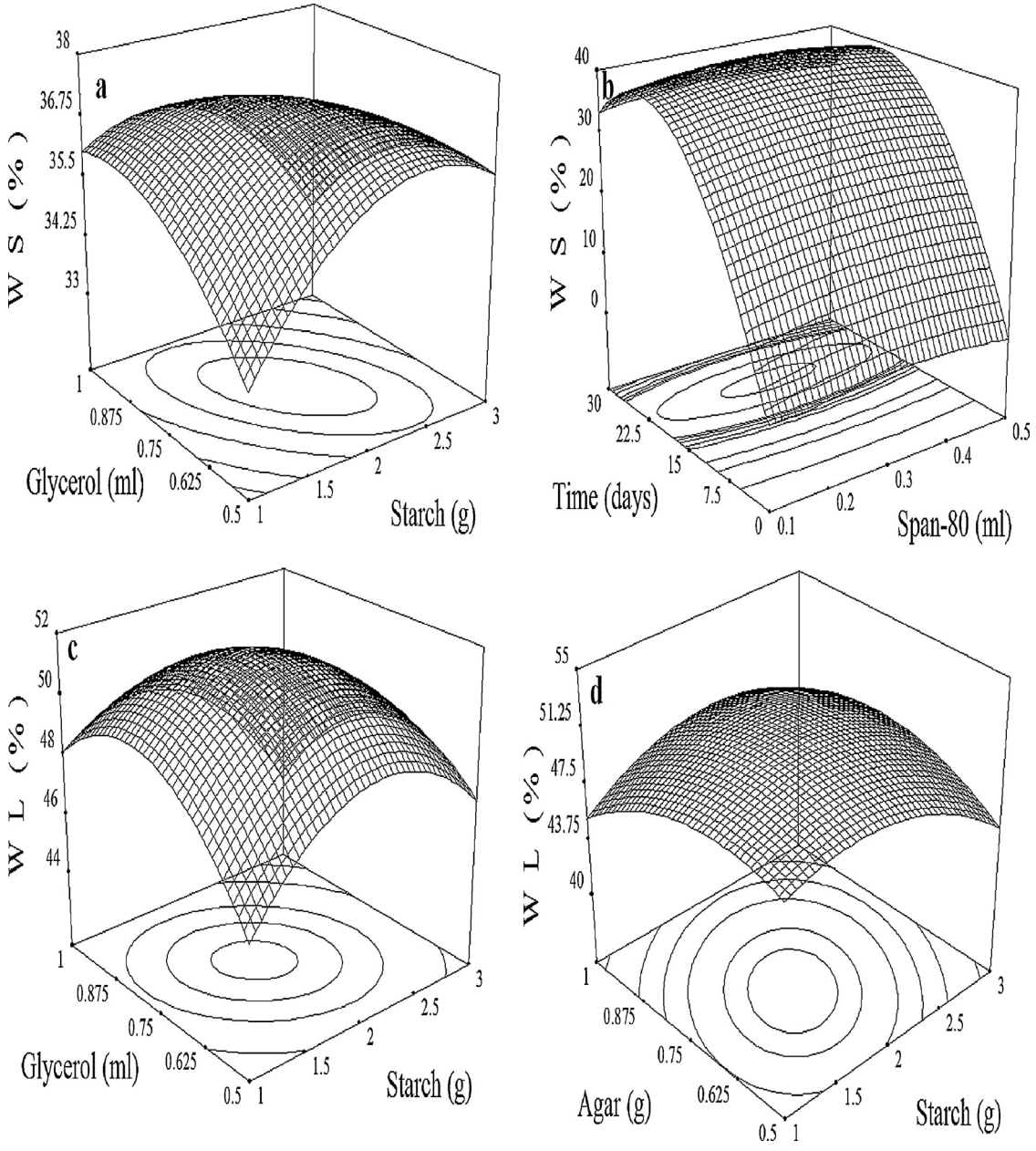

Fig. 1. Effect of process variables on WS and WL.

3. Results and discussion

3.1. Effect of process variables

Soil microbial flora constituted a mixed microbial population (including bacteria, fungi and protozoa) which may act synergistically during degradation of tapioca starch based films and reproduce under naturally occurring conditions. The experiments were carried out up to 30 days and the results are presented in Table 1. However beyond 30 days, samples could not be easily recovered due to their macroscopic deterioration.

3.1.1. Water sorption

A rapid water sorption was observed for all the specimens within the first few days of soil burial time. The starch and plasticizer (glycerol) more intensively increases the water absorption characteristics of the specimen (Fig. 1a) due to the strong hydrophilic character of the materials. The increase in the water absorption of the polymeric matrix facilitates the instability of the intermolecular interactions, causing its disintegration and consequently enhanced the solubility (Carvalho et al., 2009). Water absorption was dropped as burial time is increased, owing to the fact that some starch particle was leached away from the specimen (Ke, Sun, & Seib, 2003). The presence of span80 increases the affinity toward water by reducing the number of polar groups available to interact with water molecules and increases the water sorption of the films at later stages of degradation (Fig. 1b). The hydrophobic character of the span-80 decreases the number of O—H bonds and the presence of aliphatic groups, which changes the film structure leading to the formation of film with less solubility in water.

3.1.2. Weight loss

Weight loss of films in soil degradation could be taken as an indicator of degradation. Weight loss of films was noticed and the results are shown in Table 1. High concentration of starch and glycerol increases the weight loss of the films during soil degradation

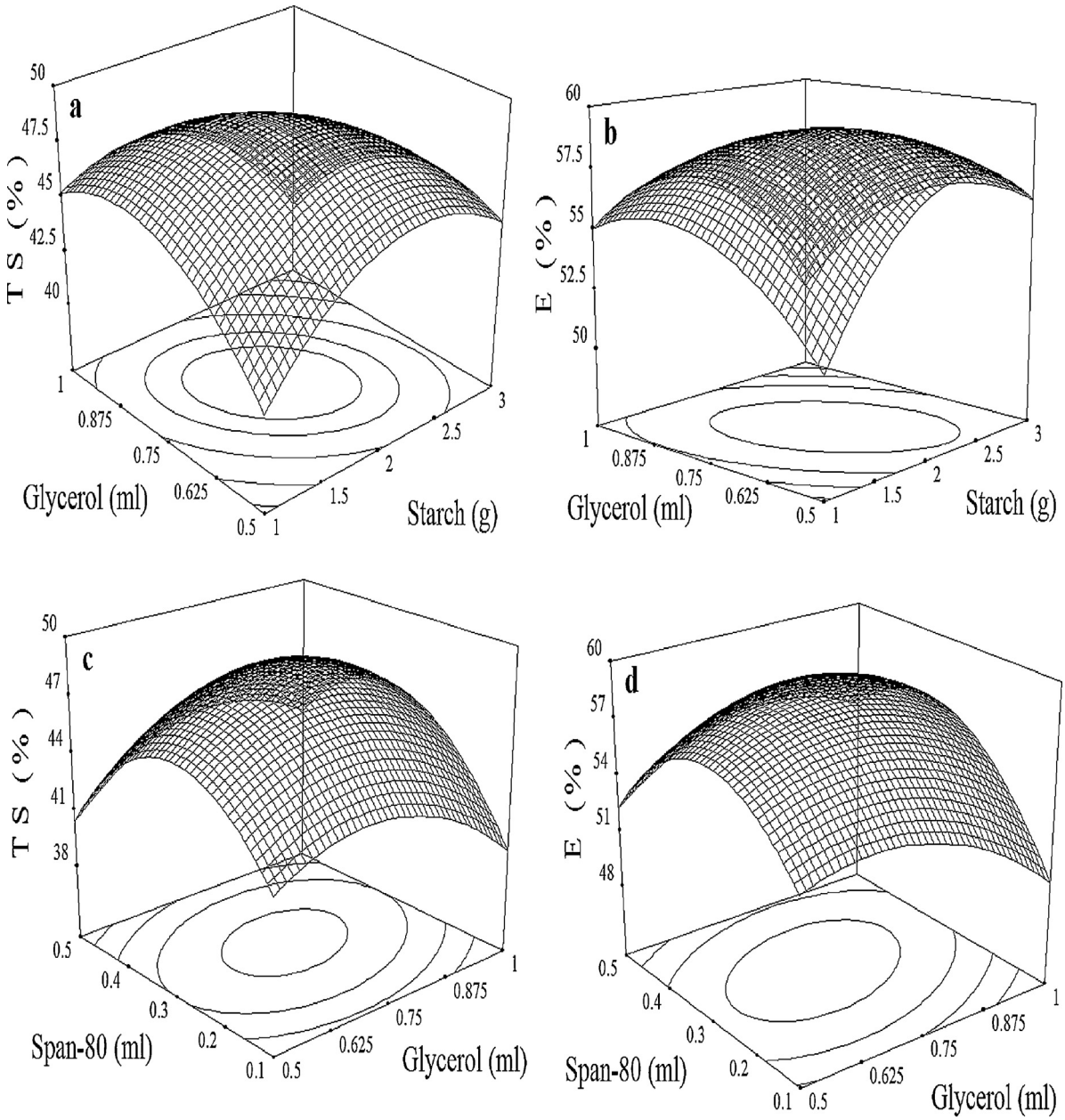

Fig. 2. Effect of process variables on the loss of mechanical properties.

of films (Fig. 1c) because both materials are hydrophilic, favors the absorption of water, increases the water activity of films and promotes the growth of microorganisms. The microorganisms which are present in the soil utilize the tapioca starch as a sole source of carbon resulting in partial degradation of films. Lopez-Llorca and Valiente (1993) showed degradation of the starch granules on the films and rapid colonization of microorganisms in the films which was buried in a forest soil. Glycerol would eventually be adsorbed by soil or passed through the cell membrane, being metabolized by microbes and increased the weight loss of the films. Characteristically, agar was sulfated polysaccharide. The presence of charged groups resulted in more extended chains with a higher hydrophilicity. So the presence of agar in the formulation, which was more hydrophilic than starch, increased the hygroscopic characteristics of the films and promotes the growth of microorganisms during degradation and increases the weight loss of the films at initial stages and then decreased (Fig. 1d).

3.1.3. Mechanical property

Loss in mechanical properties is the most relevant practical criterion in determining degradation of films. Films in contact with soil over a long period were expected to depict loss in mechanical properties. The hydrophilic nature of starch and glycerol has more susceptibility for bacterial and fungal degradations in soil (Singh & Sharma, 2008) and caused the reduction in mechanical properties of tapioca starch based films (Fig. 2a and b). Starch in the films allows water absorption and provide suitable conditions for microbial colonization and degradation of starch and esters resulting in the disintegration of films and reduced the mechanical properties of the films (Vijaya & Reddy, 2008). Wool et al. (2000) studied the degradation of polymer-starch composites in the soil and clearly showed the colonization of microorganisms within channels of the matrix that has been initially occupied by the starch. The addition of agar increased the moisture content of the film by absorbing water during degradation which may reduce the molecular weight of the

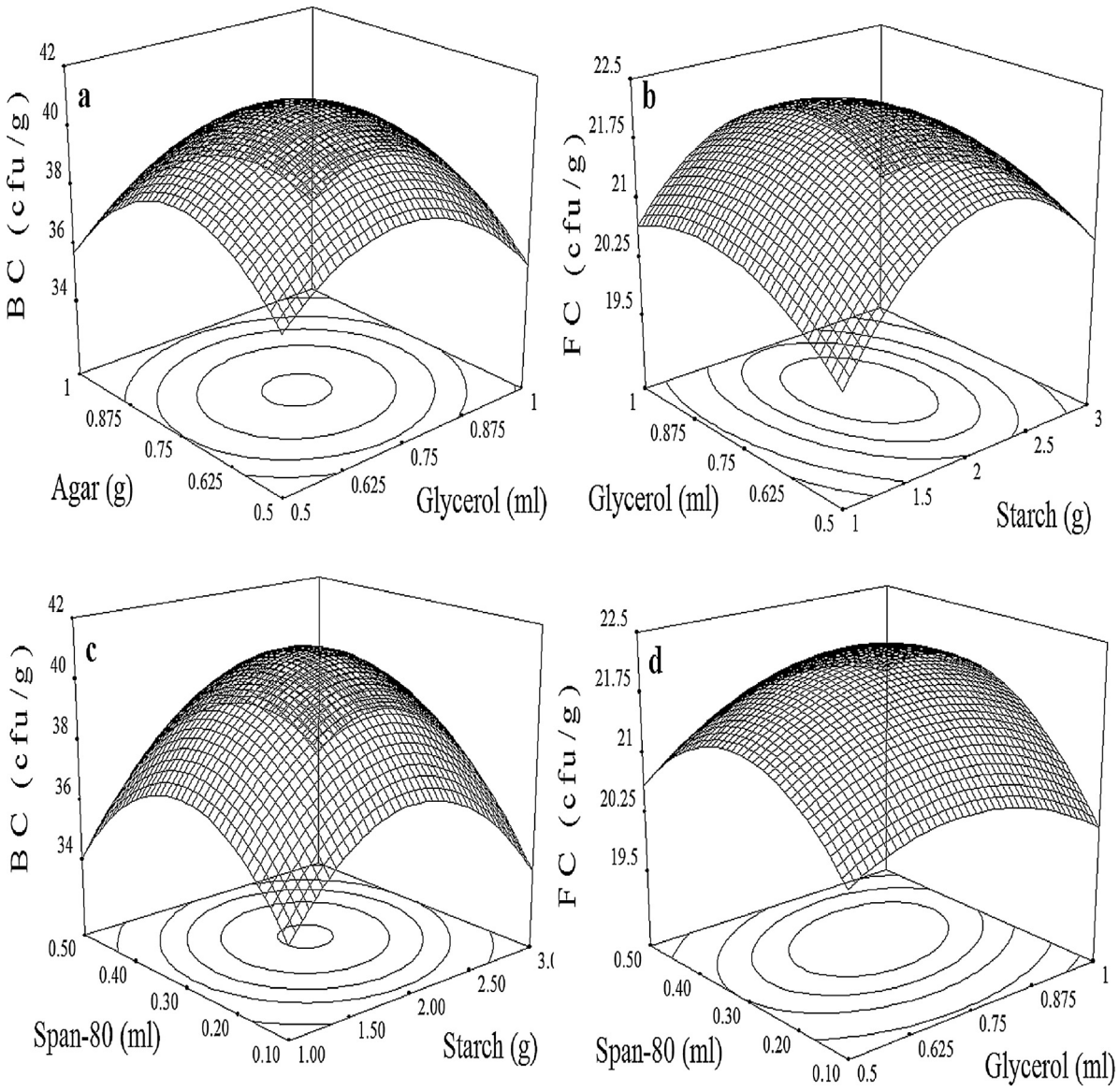

Fig. 3. Effect of process variables on BC and FC.

agar and increase the loss of mechanical properties (Freile-Pelegrín et al., 2007). The plasticizing effect of the surfactant (Span-80) increases the free volume between the adjacent chains of starch and making the structure more fragile, leading to the increased reduction in the mechanical properties of the film (Fig. 2c and d).

3.1.4. Microbial count

The microorganism quantified from the samples collected from the soil and the results showed the presence of bacteria and fungi in large number. The number of bacteria ranged from 30×10^6 to 43×10^6 CFU/g of samples. While the fungi ranged from 18×10^3 to 23×10^3 CFU/g of samples (Table 1). The average number of bacteria associated with the degradation of tapioca starch based films was found to be 29.76×10^6 and for fungi 16.93×10^3 respectively. The microbial growth was depending on the availability of water and nutrient content of materials. The glycerol increases the global water content of the material and then the swelling of the material, which give rise to an increase of the transport phenomena inside the material and promotes the growth of the microorganisms (Fig. 3a and b). On the other hand, microorganisms present in the soil hydrolyzed the starch content of the films and grown rapidly. The surfactant may facilitate the presence of glycerol between the starch chains, increasing their mobility and increased the microbial growth (Fig. 3c and d).

3.1.5. Microorganisms associated with soil degradation

To determine the biodegradability of films, it is important to identify the microorganisms associated for the degradation of the films in the soil condition. The microorganisms associated with the films were quantified and identified from the samples recovered from soil. The predominant bacterial species identified from the samples were *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp. and *Moraxella* sp. Among the fungal species identified was *Aspergillus* Sp. and *Penicillin* Sp. These microorganisms utilize starch film as a sole carbon source resulting in partial degradation of films. Similar types of microorganisms were reported earlier which is associated with films buried in the soil and have the ability for degrading polymers (Orhan, Hrenović, & Büyükgüngör, 2004; Norman, Frontera-Suau, & Morris, 2002; Kathiresan, 2003; Tadros, Noureddini, & Timm, 1999).

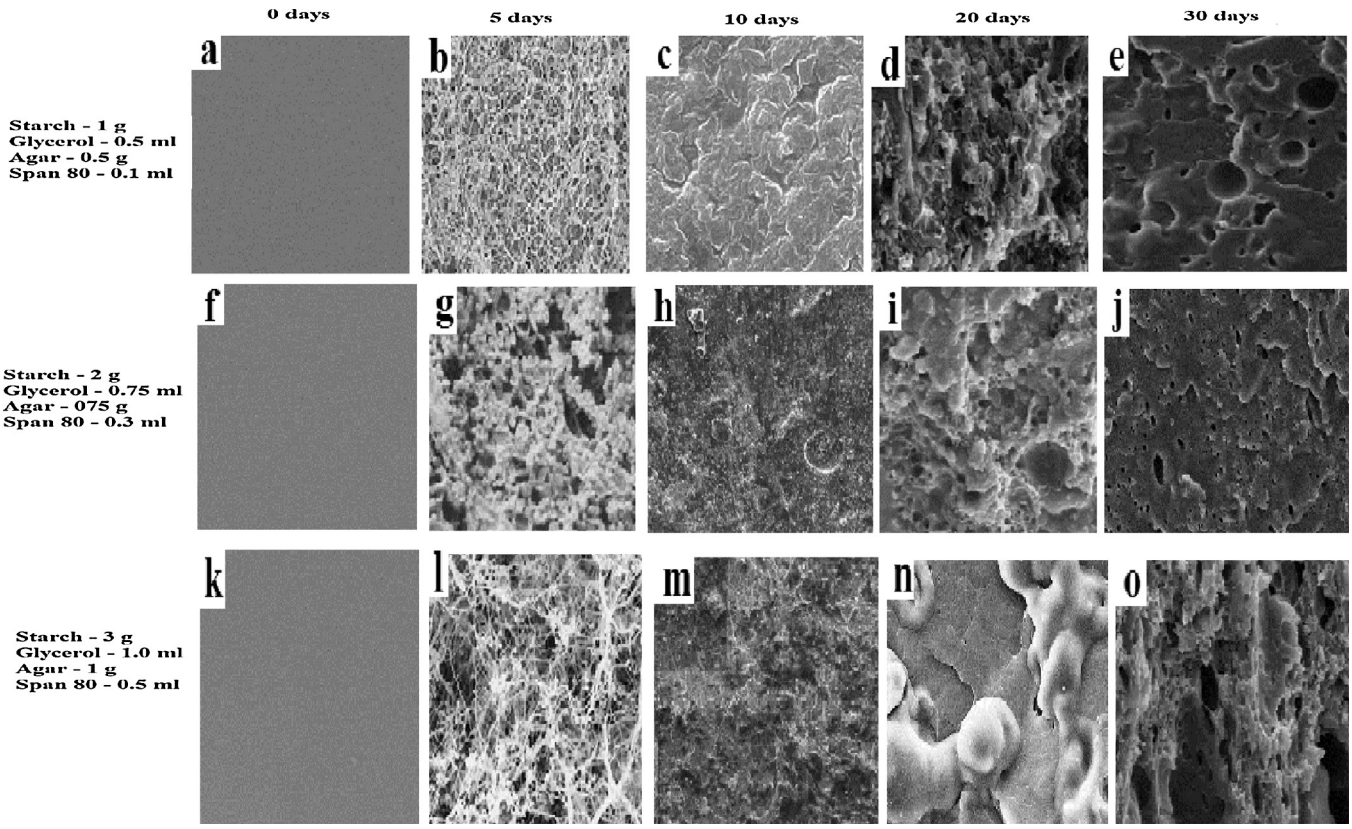

Fig. 4. Evolution of biodegradation of the samples under indoor soil conditions by SEM.

3.2. Morphology analysis

The morphological changes due to the incubation in soil were visualized from the collected specimens of materials at different degradation times (before and after) by SEM micrographs and it is shown in Fig. 4. The starch films surface was smooth before exposing to soil burial (Fig. 4a, f and k). During exposure of the samples to biodegradation, microbial biofilm began to be formed with a large number of cells growing on polymeric materials within 5 days of incubation (Fig. 4b, g and l). The starch films were degraded by both bacterial and fungal strains (Iovino, Zullo, Rao, Cassar, & Gianfreda, 2008). The presence of filaments of microorganisms on the film surface agrees well with the fact that fungi are ubiquitous and extremely effective in their biodegradation effect at different moisture regimes. As the degradation proceeded, the fungi and bacteria became very active with absolute disappearance of smooth composite matrix surface (Fig. 4c, h and m). The SEM pictures also revealed that the surface of the samples subjected to microorganisms was significantly eroded after 10 days (Fig. 4d, i and n). After 10 days, random degradation caused cavities with large holes on the surface of the films. So, the interior part of the blend was exposed with the surface completely after 20 days biodegradation (Fig. 4e, j and o). These observations may suggest that this material could be bio-assimilated by mixed soil consortia which may act in a synergetic way and promoting the degradation.

3.3. Experimental design analysis

Experimental data was obtained according to the Box–Behnken experimental design in order to study and investigate the individual and interactive effect of the process variables (tapioca starch, glycerol, agar, span80 and burial time) on the soil burial degradation properties (WS, WL, TS, E, BC and FC) of tapioca starch based films and the results are exhibited in Table 1. The experimental data was analyzed and fitted to the various models (linear, interactive (2FI), quadratic and cubic) and it is observed that, linear and interactive (2FI) models showed lower R^2, adjusted R^2, predicted R^2 and also high p-values, when compared with quadratic model. Cubic model was found to be aliased. Therefore the quadratic model incorporating linear, interactive and quadratic terms was chosen to describe and study the effect of process variables on the soil burial degradation properties of the tapioca starch based films.

3.4. Model development and statistical analysis

A second-order polynomial equation was fitted to the experimental data to develop mathematical model, which will help to predict the soil burial properties of different sets of combinations of five process variables on the responses (Prakash Maran, Manikandan, Vigna Nivetha, Dinesh, 2013; Thirugnanasambandham, Sivakumar, & Prakash Maran, 2013). Six empirical models were developed in order to understand the interactive correlation between the responses and process variables. The final model obtained in terms of coded factors is given below

$$\begin{aligned} WS = {} & 37.24 + 0.021X_1 + 0.00063X_2 - 0.59X_3 + 0.2X_4 + 16.81X_5 \\ & -1.29X_1X_2 + 1.09X_1X_3 + 0.42X_1X_4 - 1.26X_1X_5 + 0.21X_2X_3 \\ & +0.68X_2X_4 + 0.14X_2X_5 + 0.1 \quad X_3X_4 - 0.61X_3X_5 - 0.36X_4X_5 \\ & -1.52X_1^2 - 0.96X_2^2 - 1.16X_3^2 - 1.94X_4^2 - 19.03X_5^2 \end{aligned} \quad (4)$$

Table 2
ANOVA and significance of regression coefficients.

Source	DF	WS (%)		WL (%)		TS (%)		E (%)		BC (cfu/g)		FC (cfu/g)	
		RC	*p* Value	RC	*p* Value	RC	*p* Value	RC	*p* Value	RC	*p* Value	RC	*p* Value
Model	20	37.24	<0.0001	51.64	<0.0001	48.68	<0.0001	59.07	<0.0001	41.00	<0.0001	22.17	<0.0001
X_1	1	0.02	0.9516	−0.61	0.4003	−0.41	0.5637	0.0081	0.9928	0.31	0.4874	0.13	0.5169
X_2	1	0.00063	0.9985	−0.08	0.9135	−0.07	0.9168	−0.51	0.5675	−0.44	0.3332	0.13	0.5169
X_3	1	−0.59	0.0900	−0.58	0.4278	−0.79	0.2707	0.25	0.7806	−0.44	0.3332	−0.31	0.1128
X_4	1	0.20	0.5525	0.49	0.5008	0.42	0.5578	0.86	0.3395	0.38	0.4057	0.31	0.1128
X_5	1	16.81	<0.0001	22.35	<0.0001	20.84	<0.0001	26.41	<0.0001	17.56	<0.0001	10.13	<0.0001
X_{12}	1	−1.30	0.0656	−1.27	0.3844	−2.09	0.1467	−2.32	0.2027	−0.50	0.5779	−0.25	0.5169
X_{13}	1	1.09	0.1184	1.34	0.3583	2.93	0.0461	2.49	0.1717	3.00	0.0024	0.75	0.0597
X_{14}	1	0.43	0.5332	−0.19	0.8984	−0.17	0.9069	−2.89	0.1156	0.50	0.5779	−0.50	0.2005
X_{15}	1	−1.26	0.0727	−1.67	0.2561	−1.51	0.2901	−2.93	0.1112	−0.75	0.4057	−0.50	0.2005
X_{23}	1	0.21	0.7574	0.15	0.9162	−0.36	0.8001	−0.60	0.7376	0.00	1.0000	0.00	1.0000
X_{24}	1	0.68	0.3234	0.80	0.5809	1.18	0.4083	1.53	0.3952	0.75	0.4057	0.25	0.5169
X_{25}	1	0.14	0.8368	−0.21	0.8875	0.0001	1.0000	−0.57	0.7492	−0.50	0.5779	0.00	1.0000
X_{34}	1	0.10	0.8830	−0.24	0.8671	−0.11	0.9379	−0.10	0.9554	−0.25	0.7803	0.25	0.5169
X_{35}	1	−0.62	0.3692	−1.53	0.2964	−1.11	0.4354	0.28	0.8779	−0.50	0.5779	−0.25	0.5169
X_{45}	1	−0.36	0.5997	−0.70	0.6323	−0.83	0.5578	−1.53	0.3959	−1.00	0.2701	0.25	0.5169
X_1^2	1	−1.52	0.0026	−2.67	0.0111	−3.12	0.0029	−3.50	0.0073	−3.31	<0.0001	−1.21	<0.0001
X_2^2	1	−0.96	0.0458	−2.66	0.0113	−2.75	0.0075	−2.31	0.0659	−2.48	0.0004	−0.54	0.0456
X_3^2	1	−1.16	0.0175	−2.35	0.0233	−2.56	0.0121	−1.53	0.2133	−2.81	<0.0001	−1.29	<0.0001
X_4^2	1	−1.94	0.0002	−4.34	0.0001	−4.81	<0.0001	−4.28	0.0015	−3.23	<0.0001	−0.96	0.0010
X_5^2	1	−19.03	<0.0001	−26.28	<0.0001	−24.54	<0.0001	−29.75	<0.0001	−20.48	<0.0001	−11.04	<0.0001
R^2		0.994		0.986		0.985		0.984		0.991		0.995	
Adj-R^2		0.990		0.975		0.973		0.972		0.984		0.991	
Pre-R^2		0.978		0.952		0.947		0.944		0.971		0.984	
CV%		4.69		7.49		7.86		7.93		5.96		4.49	
Adeq.Pre		42.52		27.94		27.24		26.25		35.59		44.76	

$$\begin{aligned}WL = {} & 51.64 - 0.61X_1 - 0.079X_2 - 0.58X_3 + 0.49X_4 + 22.35X_5 \\ & -1.27X_1X_2 + 1.34X_1X_3 - 0.19X_1X_4 - 1.67X_1X_5 + 0.15X_2X_3 \\ & +0.8X_2X_4 - 0.2X_2X_5 - 0.24X_3X_4 - 1.53X_3X_5 - 0.69X_4X_5 \\ & -2.67X_1^2 - 2.66X_2^2 - 2.35X_3^2 - 4.34X_4^2 - 26.28X_5^2 \end{aligned} \quad (5)$$

$$\begin{aligned}TS = {} & 48.68 - 0.41X_1 - 0.074X_2 - 0.79X_3 + 0.41X_4 + 20.84X_5 \\ & -2.09X_1X_2 + 2.93X_1X_3 - 0.17X_1X_4 - 1.51X_1X_5 - 0.36X_2X_3 \\ & +1.17X_2X_4 + 0.0001X_2X_5 - 0.11X_3X_4 - 1.11X_3X_5 - 0.83X_4X_5 \\ & -3.12X_1^2 - 2.75X_2^2 - 2.56X_3^2 - 4.81X_4^2 - 24.54X_5^2 \end{aligned} \quad (6)$$

$$\begin{aligned}E = {} & 59.07 + 0.0081X_1 - 0.51X_2 + 0.25X_3 + 0.86X_4 + 26.41X_5 \\ & -2.32X_1X_2 + 2.49X_1X_3 - 2.89X_1X_4 - 2.93X_1X_5 - 0.6X_2X_3 \\ & +1.53X_2X_4 - 0.57X_2X_5 - 0.1X_3X_4 + 0.28X_3X_5 - 1.53X_4X_5 \\ & -3.5X_1^2 - 2.31X_2^2 - 1.53X_3^2 - 4.28X_4^2 - 29.75X_5^2 \end{aligned} \quad (7)$$

$$\begin{aligned}BC = {} & 41 + 0.31X_1 - 0.44X_2 - 0.44X_3 + 0.37X_4 + 17.56X_5 \\ & -0.5X_1X_2 + 3X_1X_3 + 0.5X_1X_4 - 0.75X_1X_5 + 0.0001X_2X_3 \\ & +0.75X_2X_4 - 0.5X_2X_5 - 0.25X_3X_4 - 0.5X_3X_5 - 1X_4X_5 \\ & -3.31X_1^2 - 2.48X_2^2 - 2.81X_3^2 - 3.23X_4^2 - 20.48X_5^2 \end{aligned} \quad (8)$$

$$\begin{aligned}FC = {} & 22.17 + 0.12X_1 + 0.12X_2 - 0.31X_3 + 0.31X_4 + 10.13X_5 \\ & -0.25X_1X_2 + 0.75X_1X_3 - 0.5X_1X_4 - 0.5X_1X_5 + 0.0001X_2X_3 \\ & +0.25X_2X_4 + 0.0001X_2X_5 + 0.25X_3X_4 - 0.25X_3X_5 + 0.25X_4X_5 \\ & -1.21X_1^2 - 0.54X_2^2 - 1.29X_3^2 - 0.96X_4^2 - 11.04X_5^2 \end{aligned} \quad (9)$$

Pareto analysis of variance (ANOVA) was used to analyze the experimental data and the results are listed in Table 2. The higher model *F*-value (222.44 for WS, 88.12 for WL, 80.64 for TS, 78.54 for E, 140.16 for BC and 242.97 for FC) and the associated lower *p*-values ($p < 0.0001$) demonstrated the significance of the developed models and also indicates that most of the variation in the responses could be explained by the regression equations (Thirugnanasambandham et al., 2013a). The high value of R^2 (0.994 for WS, 0.986 for WL, 0.985 for TS, 0.984 for E, 0.991 for BC and 0.995 for FC), adj-R^2 (0.989 for WS, 0.975 for WL, 0.973 for TS, 0.972 for E, 0.984 for BC and 0.991 for FC) and pre-R^2 (0.977 for WS, 0.952 for WL, 0.947 for TS, 0.944 for E, 0.972 for BC and 0.984 for FC) clearly demonstrated that, the form of the model chosen to represent the actual relationship between the response and independent variables is well correlated and accurate. Low values of coefficient of variance (4.69 for WS, 7.49 for WL, 7.86 for TS, 7.93 for E, 5.96 for BC and 4.49 for FC) displayed the high degree of precision and good reliability of the conducted experiments (Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013). In this study, the adequate precision (signal to noise ratio) was found to be >26 for all responses, which indicates the best fitness of the developed models (Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013).

4. Conclusions

Mixed undefined microbial population present in the soil microflora was used as the degrading medium in the present study and as it can be considered a realistic approach to the biodegradation process in natural environments. Degradation during indoor soil burial experiments of starch based composite films were analyzed using five factors, three level Box–Behnken response design. From the results, it was observed that, the water intake promotes the entry of microorganisms in to the films, due to the higher water availability within the material, and the composites wee potentially degraded in the natural environment. The soil microorganism utilizes the starch films as a source of energy for their growth, the weight of the films was decreased and consequently the mechanical property of the film was also

reduced. The microorganisms associated with the degradation of films were quantified and identified. SEM chronological images showed the biodegradation indicators such as fractures, breaches, cavities, and holes on the film surface. From the experimental data, second order polynomial models were developed for the responses. The results of the study clearly demonstrated that, tapioca starch based films are edible and biodegradable renders them good candidates to replace non-biodegradable synthetic plastic systems in the consideration of food packaging systems.

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