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Research Article

Biodegradability Determination of Soy Protein Concentrate -Corn Starch-C30B Nanocomposites

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Abstract: In this study the biodegradability of Soy protein Concentrate (SPC) crosslinked with Corn starch (CS) and Cloisite 30B (C30B) has been discussed threadbare. The nanocomposites were prepared by employing the solution casting method. The physical and chemical properties of nanocomposites SPC-CS/C30B were investigated using XRD, FTIR, SEM, TGA and tensile strength. The biodegradation properties of samples were measured by micro-organic culture testing, enzymatic absorbance in water, soil burial and compost testing in both laboratory and field conditions. SPC-CS/C30B nanocomposites exhibited favourable physical properties. Additionally it was observed that when the percentage of C30B was increased up to 4% with SPC/CS, the tensile strength was also increased up to 45.67%. The morphology study of SPC-CS/C30B was found exfoliated form with porous structure.

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1. Introduction

Around the Globe the volume of production of thermoplastics is around 150 million tons and is expected to reach 340 million tons by 2025AD. More than one third of commodity plastics (PE, PS, PAN, PP, PIB and PET) are mainly used for packaging and for short life products. The per capita consumption of plastics in India is very low, about 5 kg in comparison to 150kg in USA, 60 kg, in Japan and 20 kg in Europe. In India commodity plastics account for about 85 per cent of the total consumption of plastics (Richard et al., 2009 ; Shaxson et al., 2009; Talsness et al., 2009; Teuten et al., 2007; Thompson et al., 2005; Wagner et al., 2009; Guang-Hua Zhang et al., 2007).

Biodegradable plastics made from plant-based materials have been available for many years. Due to their high cost, they never replace traditional non-degradable plastics. In the most general sense "biodegradable" means that a substance is able to be broken down into other innocuous substances, with a significant change of chemical structure, by the activities of living organisms and is therefore unlikely to persist in the environment (Guang-Hua Zhang et al., 2007).

Biodegradable plastics are a new generation of polymers emerging in the market. Biodegradable plastics have an expanding range of potential applications, and are driven by the growing use of plastics in packaging and the perception that biodegradable plastics are 'environmental friendly', their use is predicted to increase. However, issues are also emerging regarding the use of biodegradable plastics and their potential impact on the environment and effects on established recycling systems and technologies. There is an extensive range of potential applications. Some of these include: film including over wrap, shopping bags, waste and bin liner bags, composting bags, mulch film, silage wrap, landfill covers, packaging,

bait bags and cling wrap, flushable sanitary products, sheet and non woven packaging, bottles, planter boxes and fishing nets, food service cups, cutlery, trays, and straws (Lebreton et al., 2017; Ren et al., 2003; Conrad et al., 2005; Conrad et al., 2005).

It is well documented that biopolymers have attracted more attention and interest due to the imminent petroleum crisis, increase in environmental pollution from consumed petroleum based materials and their environmental friendly nature. Biopolymers from various natural botanical resources, such as starch, protein and cellulose have been regarded as alternative materials to petroleum plastics because they are abundant, renewable, inexpensive, eco-friendly and biodegradable (Steinbüchel et al., 1992; Scheller et al., 2005; Kumar et al., 2004; Conrad et al., 2005). In all of the protein resources, soy protein has been regarded as a renewable biopolymer and a potential source, not only can be used as biodegradable plastics, textile fibers, biodegradable membranes and adhesives, but also can be acetylated, transglutaminase-treated and blended with organic or inorganic nanoparticles (Conrad et al., 2005). There is one kind of soy protein named soy protein concentrate (SPC) with more than 70% protein and 18 diverse amino acids. The ultracentrifugal pattern of SPC generally shows a wide range of molecular weights (8–600 kDa), with four major fractions identified as 2S (20%–22%), 7S (37%), 11S (31%–40%), and 15S (10%–11%). It is noticeable that plastics made from SPC possess good biodegradability but have poor flexibility (Jong et al., 2006). Dry SPC is a rigid material so plasticizers are usually used to overcome the brittleness of soy protein plastics, but unavoidably it leads to a significant decrease in mechanical properties (Steinbüchel et al., 1992; Scheller et al., 2005; Kumar et al., 2004)..

Hence, the aims of this investigation are to investigate the effects of SPC-CS/C30B nanocomposite films. The purpose of this work is to explore the possibility of preparing SPC-CS/C30B nanocomposites using solution casting method. The prepared SPC-CS/C30B bionanocomposites were characterized, and the relationship between the structure and mechanical properties was analyzed. Moreover, the thermal stability was also investigated.

2. Materials and methods

Corn starch (CS) was purchased from HiMedia. Soy protein concentrate (SPC) was obtained from Hindustan Scientific Traders, Odisha, India. Cloisite 30B was purchased from Southern Clay Products, USA. All other chemicals and reagent were obtained from HiMedia and Sigma-Aldrich and used as the analytical grade.

Cloisite30B was added in the blended mixture as 1%, 2%, 3%, 4% and 5%. The mixture was stirred at 37⁰c for 8 hours up to the formation of a homogenate matrix. Further, the blended homogenized polymer nanocomposite (SPC-CS/C30B) was poured in a petridish and dried at 37⁰C temperatures for three days. Then the sample was ready for characterization in table .1. The thicknesses of the films were about 0.09 mm. The samples were collected in desiccators before characterization.

3. Characterization

3.1. Fourier transform Infrared Spectroscopy (FTIR):

FTIR for SPC-CS and SPC-CS/C30B films were obtained on a Nicolet 5-5X C FTIR spectrometer by diffuse reflectance.

Table.1. Sample preparation of SPC-CS/C30B nanocomposite

Sample	SPC	CS	C30B
SPC-CS/ (100/0)	100	0	-
SPC -CS/ (90/10)	90	10	-
SPC -CS/ (80/20)	80	20	-
SPC -CS/ (70/30)	70	30	-
SPC -CS/ (60/40)	60	40	-
SPC-CS/ (50/50)	50	50	-
SPC-CS/C30B (60/40-1%)	60	40	1%
SPC -CS/C30B / (60/40-2%)	60	40	2%
SPC -CS/C30B / (60/40-3%)	60	40	3%
SPC -CS/C30B / (60/40-4%)	60	40	4%
SPC -CS/C30B / (60/40-5%)	60	40	5%

2.1. Preparation of Blend Films

SPC was dried for about 3 hrs at 65°C (using a vacuum oven). The mixture of SPC and CS solution were prepared with different proportion such as (SPC: CS) 100/0, 80/20, 70/30, 60/40, 0/100 (w/w) respectively. The mixtures of blending polymer were stirred at 37⁰C for 4 hours to acquire homogeneous solutions in presence of minimum amount of hot water and glycerol. Additionally,

3.2. X-ray Diffraction (XRD)

D8 Advance Diffractometer (Bruker, U.S.A.) equipped with a CuK α radiation source (λ = 0.154 nm) was utilized for X-ray diffraction. The diffraction data were obtained from 2θ = 1°–80°.

3.3. Scanning Electron Microscopy (SEM)

Liquid nitrogen was used for freezing the SPC-CS and SPC-CS/C30B sheets and then snapped quickly, and then the fractured surface was

sputtered with gold and investigated with a Scanning electron microscope instrument (JSM-5900LV) using an acceleration voltage of 20 kV.

3.4. Mechanical Properties

The tensile properties of the sample were incorporated on a universal testing machine (CMT4104, Shenzhen SANS Test Machine Co. Ltd, CIPET, and Odisha) with a tensile rate of 5 mm/min.

3.5. Thermogravimetric Analysis (TGA)

Perkin Elmer Thermogravimetric Analyzer (TGA-7) was used to analyze the TGA of the samples. The test samples (about 3 mg) were allowed to be heated at 50°C to 500°C at a heating rate of 20 K/min.

3.6. Micro-Organic Culture Test

Microbial measurements were based on the dilution method of plate counting. Suspensions of soil collected from a garden which had been covered with the SPC-CS/C30B films for the cultivation. The properties for the soil were as follows: The soil pH was 6.0, the soil carbon was 25.25 g/kg, total nitrogen was 1.15 g/kg, total phosphorus was 0.75 g/kg, and total potassium was 16.24 g/kg. The test was carried out over a period of 60 days from June 1, 2017 to August 1, 2017. The SPC-CS/C30B film was used for the micro-organic culture test by laying the film at the bottom of the culture dish. Beef-protein agar medium was used to culture bacteria at 37°C for 48 h, Martin substratum was used to culture fungi at 28°C for 72 h, and Gauze's No. 1 synthetic medium was used to culture Actinomycetes at 28°C for 72 h. The measurement of microorganism was performed in triplicate.

3.7. Enzymatic Testing

A compound of DH₂O, α -amylase, and β -amylase were put in a sterile conical flask with specific ratio. Then the sample was cut into 4 X 4 cm square from the SPC-CS and SPC-CS/C30B. The flasks were incubated in a shaking incubator at a

rate of 200 rpm for 48 hours at 40°C. After an appropriate time to remove the enzymatic mixture from sample surface, then samples were cleaned with distilled water and dried in desiccators under vacuum for 24 hours before being weighed. The degree of enzymatic degradation [DED (%)] was measured using the following Equation 1

$$DED(\%) = \frac{W_1 X W_2}{W_1} \times 100$$

Equation 1

Where W_1 : Dry weight of sample after the enzymatic treatment

W_2 : Initial dry weight of the sample.

3.8. Biodegradability through Soil and Compost Burial test

The samples were buried separately in soil and compost. First two different pots (capacity 10 litres of each) filled with soil and compost was used. Then prepared SPC-CS and SPC-CS/C30B were cut into 25 × 40 mm pieces buried in the soil and compost at a depth of 15 cm in laboratory condition. The weight loss of the sample was indicating rate of degradation of by soil and compost burial test method. The following formula (equation 2) was utilized for calculating the weight loss of the sample in every seven days from the starting day.

$$\text{Weight Loss}(\%) = \frac{W_i X W_d}{W_i} \times 100$$

Equation 2

Where W_d : Dry weight of the sample after being washed with distilled water

W_i : Initial dry weight of the sample

Figure.1. FTIR of SPC, CS, C30B, SPC/C30B and CS-SPC/C30B

4. Result

4.1. FTIR

Fig.1 shows the FTIR spectra of SPC, CS, C30B, SPC/C30B, and CS crosslinked SPC/C30B. In figure 1a, Pure SPC shows peaks around 1632 cm^{-1} , 1539 cm^{-1} , and 1238 cm^{-1} correspond to amide I (C=O stretching), amide II (N-H bending) and amide III (C-N and N-H stretching) of SPC. In SPC/C30B blended films, the characteristic peaks

at 527 cm^{-1} (Al-O-Si deformation), 1042 cm^{-1} (Si-O in-plane stretching), 1069 cm^{-1} (C-O stretching), 1242 cm^{-1} (coupled C-N and C-O stretching), 1552 cm^{-1} (C-NH vibration), 1722 cm^{-1} (carbonyl stretching). The above result characteristic peaks were shifted in sample SPC/C30B with comparing SPC. It is indicating that the polymer chains have

intercalated into C30B (Salze et al., 2010; Dawson et al., 1988).

Pure CS shows peaks at 893 and 1156 cm^{-1} corresponding to saccharide structure, (1658 and 1322 cm^{-1} 1383 and 1424 cm^{-1} (CH_3 symmetrical deformation mode), 1030 and 1080 cm^{-1} (C-O stretching vibration) and 2800 and 2900 cm^{-1} (C-H stretch vibrations) (Yoshimura et al., 1998; Chen et al., 2013)..

In case of CS crosslinked SPC/C30B film; there are two distinct peaks at 1407 and 1544 - 1563 cm^{-1} . The characteristic peak at 1545 - 1561 cm^{-1} (symmetric deformation of NH_3 and ionization of primary amino groups in the acidic medium) whereas the peak at 1407 cm^{-1} (carboxylic acid), 1700 - 1725 cm^{-1} (carboxylic acid), 1211 - 1310 cm^{-1} (C=H vibration).

In fig.2. C30B shows peak at $2\theta = 4.78^\circ$. In the case of 4 wt% C30B in SPC/C30B, this peak shifts to $2\theta = 4.35^\circ$ which indicates a little increase of spacing due to intercalation form in between C30B and SPC chains. However, in same time and same condition when the percentage of C30B was increased with 5wt%, the peak position was shifted to lower angles and decrease peak intensity. This is confirmed that partial exfoliation of C30B in SPC matrix has taken place. It also observed that in the higher percentage of C30B loading i.e. 5wt%, no significant change was observed (Thymi et al., 2005; Mendes et al., 2016; Yoshimura et al., 1998). In CS crosslinked SPC/C30B, peak of SPC/C30B moves to a lower angle from $2\theta = 4.35^\circ$ to $2\theta = 4.23^\circ$. The increase of interlayer spacing for CS-SPC/C30B indicates the intercalation of CS into interlayer of SPC/C30B, and the formation of CS-SPC/C30B structure.

4.2. XRD

Figure.2. XRD of C30B (a), SPC, CS, C30B, SPC/C30B and CS-SPC/C30B (b)

Figure.3. SEM of SPC, SPC/C30B and CS-SPC/C30B

4.3. SEM

The SEM images of the abraded surfaces of SPC, SPC/C30B and CS cross linked SPC/C30B are shown in fig. 3. The fractured surface of SPC with 4% of C30B films was smoother and partially aggregated (Huang, 2012). When CS was incorporated, the surfaces of films CS-SPC/C30B was smoother than film SPC/C30B (60:40), as a result of the reaction in between CS-SPC/C 30B. It indicates that the addition of both SPC/C30B and CS crosslinked SPC/C30B can increase the toughness of the nanocomposites considerably

(Thymi et al., 2005; Mendes et al., 2016; Yoshimura et al., 1998).

4.4. Thermal Properties of the CS-SPC/C30B nanocomposite

In table-2 shown that thermal properties of SPC crosslinked with CS and C30B nanocomposite films. Here the weight loss was measured in two stages, first decomposition stage range from 100 to 200⁰C and second decomposition stage range from 250 to 350⁰C. The primary degradation temperature of the first Stage for 2% C30B and 3% C30B was

not significantly different compared with SPC. However, the degradation temperature of 4% C30B in the first Stage was greatly decreased. Surprisingly the temperature of the initial degradation and maximum degradation rate in the first Stage for SPC-CS/C30B was increased due to the formation of hydrogen bonds in between CS and SPC. The initial degradation temperature in the second stage of sample 2% C30B, 3% C30B C30B was not significantly changed. From the TGA report, it was clear that the initial degradation temperature in the second Stage for SPC-CS/C30B increased significantly confirmed that the reaction occurred between SPC and CS/C30B group.

4.5. Mechanical Properties of the SPC crosslinked with CS and C30B

In fig.4 shows tensile strength of SPC-CS/C30B with different percentage of C30B. The strain-stress curves of the SPC-CS/C30B, in result the stress continuously increased with increasing of strain. From result, when C30B was increased up to 4%, the tensile strength of the SPC-CS/C30B increased near about 45.67 MPa then rate of strength was decreased at 5% C30B. This decreasing result may be caused by the aggregation of C30B, which was consistent with the test results previously mentioned. Additionally, when CS was introduced, tensile strength of the modified SPC-CS/C30B was continuously enhanced due to SPC successfully crosslinked with CS. The successful preparation of SPC-CS/C30B was indicated the hydrogen bonding formation in between CS and SPC (Kumar et al.,2010)..

4.6. Micro-Organic Culture Test

Fig.5 shows SPC/CS and SPC-CS/C30B films behaved in the micro-organic culture test. The soil microbial quantities of bacteria were richest using SPC-CS/C30B films. The SPC-CS/C30B nanocomposites films can produce more microbial biodegradation due to heat generated beneath the

films. However the increase in bacteria and actinomycetes might be beneficial for the biodegradation of this SPC-CS, and SPC-CS/C30B nanocomposites films. From result, SPC-CS/C30B is better biodegradation than SPC/CS polymer at same time with same condition (Heydari et al., 2013;YuneLi et al .,2007;Huang et al., 2006;Hanifi et al .,2014).

4.7. Enzymatic Testing

Fig. 6 shows the weight loss of the film. With increasing C30B content in the SPC-CS films, an increase in degradation was found. The degradation rate of SPC-CS film (without C 30B) was slower than that of SPC-CS films (with C30B). Similarly, at the same time, SPC cross linked with CS/C30B is higher degradation rate than SPC-CS composite. Around 57% for CS-SPC and 91% for SPC-CS/C30B weight loss after 72hours of immersion was found as the absorption of the enzymatic solution was directly proportional to the SPC content. Among the enzymes β -amylase and α -amylases, the later hydrolysis SPC and CS more rapidly than former because it breaks the SPC molecule. The hydrolysis of the SPC results is indicated that weaker interaction in between SPC-CS and C30B. So that the results reported higher weight loss in SPC-CS/C30B.

4.8. Soil Burial Test

The biodegradability and burial time (both in soil and compost) are directly proportional to each other. Almost 79% for SPC-CS/C30B (60:40:4%) of biodegradation was observed after ten weeks in the soil in fig.7a. Similarly, 85% for SPC-CS/C30B (60:40:4%) of biodegradation was observed after ten weeks in compost in fig.7b. Then the sample was continued to degrade and decompose at a slow rate because of the reduction of oxygen level and temperature in the film. In both the soil and compost, SPC-CS/C30B sample showed the highest weight loss than pure SPC. CS and C30B

have a major role in biodegradation of SPC film. The hydrolysis ability property of SPC films decreases with the increasing concentration of C30B. So it shows a strong resistance to its degradation in soil burial test. However, a 5–6% increase in the degradation rate was observed in the case of compost burial. The presence of different types of organic matter in compost helped it to decompose largely through aerobic decomposition. Compost is made up of high nutrient content and hence can be used as a very good fertilizer and soil conditioner. Probably this high level of carbohydrate and nutrients molecules present in the compost helps to degrade the sample more quickly by the microbes than soil.

5. Discussion

Biodegradable plastics derived from agricultural feed-stocks are a new generation of materials capable of reducing the environmental impact in terms of energy consumption and green-house effect in specific applications to perform as traditional plastics when in use and completely degrade within a composting cycle through the action of living organisms when engineered to be biodegradable (Ren et al.,2003; Conra et al.,2005). A green plastic from renewable resources is a challenging field of research with unlimited future prospects.

Table.2. TGA of SPC, SPC-CS/C30B nanocomposite film

Sample	T _{il} (°C)	T _{Max1} (°C)	T _{i2} (°C)	T _{Max2} (°C)
SPC	132.21	158.42	271.64	297.91
CS	123.22	147.24	254.32	276.92
SPC:CS (60:40)	136.10	177.51	264.73	295.83
SPC:CS (60:40) : C30B (2%)	137.53	164.53	277.21	302.04
SPC:CS (60:40) : C30B (3%)	127.81	156.92	278.84	304.11
SPC:CS (60:40) : C30B (4%)	138.43	177.11	288.59	303.35
SPC:CS (60:40) : C30B (5%)	146.21	178.53	288.53	297.44

Figure.4. Tensile strength of (a) SPC, CS, SPC/CS and (b) CS-SPC/C30B

Figure.5. the amount of microorganisms in soil covered by different films actinomycetes (a) and bacteria (b) between June 2, 2017 and August2, 2017.

Figure. 6. Enzymatic degradation of CS-SPC/C30B (100/0) films using α -amylase and β -amylase

Figure.7a. Weight loss of SPC-CS/C30B films after 8 weeks in soil.(b)Weight loss of SPC-CS/C30B films after 8 weeks in Compost.

Figure.8. Schematic presentation of SPC-CS/C30B nanocomposite for biodegradation

Two most important renewable resource plant-based materials such as soy protein concentrate and corn starch have been used to synthesize some biodegradable plastics using some modifiers such as Cloisite 30B (Thymi et al., 2005; Mendes et al., 2016; Yoshimura et al., 1998). The nanocomposites of soy protein were fabricated by blending it with some nanoparticles such as Cloisite 30B. The bionanocomposites were characterized using a multitude of methods such as FTIR, SEM, XRD, and TGA. In some cases the rheological properties were measured. The biodegradation of the composites and nanocomposites were also investigated. From these studies the morphology as well as the crystallinity of the green composites were evaluated since these properties are very much essential for making degradable films and molded products. From these studies it is ascertained that these renewable materials along with their nanocomposites could be used for making degradable consumer items.

Soy protein concentrate blended with corn starch and organoclay (Cloisite 30B), bio-based, eco-friendly nanocomposites, were prepared from SPC/CS (60/40 wt/wt) with organo-modified clay by solution casting. The XRD, SEM, tensile properties of the nanocomposites were investigated. X-ray diffraction and transmission electron microscopy analysis revealed that the intercalated nanocomposite is formed and the silicate layers of the clay are uniformly dispersed at a nanometer scale in the polymer matrix. There is a great enhancement in tensile and dynamic mechanical properties of the nanocomposites. Here we have drawn a scheme of the determination of biodegradation of SPC-CS/C30B (Fig. 8). Here we draw a schematically.

This study is really a good breakthrough in enhancing the concept of biodegradable polymers, for their commercial applications and multipurpose

uses. Based on the results, many small scale and medium scale industries could be developed in our country to prepare cheap degradable materials such as films, cups, plates, cutlery etc to keep the environment clean and green.

6. Conclusion

In this study, SPC-CS/C30B nanocomposite film was favourably prepared. The tensile strength of SPC-CS/C30B increased with the addition of C30B up to 4%. From XRD result, SPC-CS/C30B was completely exfoliated. Additionally the FTIR results indicated that CS was excellent crosslink material for SPC/C30B by opening ring reaction between amino groups and OH. The increased degradation rate of the sample was observed with the addition of C30B to the SPC matrix, and SPC-CS/C30B. The degradation process increases with the increase in C30B content in the SPC. It is also observed that SPC-CS/C30B is associated with higher rate of biodegradation than SPC/C30B. Thus SPC-CS/C30B film has a great deal of future promise for the potential application which will contribute to global sustainability.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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