Anaerobic Biodegradation of Urea Cross-linked Starch: Effect of Lignin on Tensile Properties

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Lignin was used as a natural filler to improve the recalcitrance of environmentally friendly biocomposites made from starch. The tensile properties of lignin-starch biocomposites prepared by lignin reinforcing of urea cross-linked starch (UcS) were investigated in this study. The amount of lignin loaded into UcS was from 5 to 20%. These various compositions were buried in a microcosm of anaerobic soil. After 7 days of burial, biodegraded biocomposites were tested for changes in tensile characteristics. Changes in biodegradation were measured by comparing them to pristine samples, which were utilised as a benchmark for estimating. Through reinforcing polymeric starch in UcS, lignin was discovered to slow down the rate of loss in tensile characteristics of composites. With increasing lignin loadings from 5 to 20%, biodegraded biocomposites showed a constant reduction in elongation at break, Young's modulus, and tensile strength. As a result, the biodegraded biocomposites' metrics exhibited a substantially slower decrease than the control biodegraded film. The reduction in tensile properties of biodegraded biocomposites was explained by a significant difference ($p < 0.05$) using a paired t-test. This study found that lignin increased the strength of UcS and reduced the loss of tensile characteristics, probably as a result of soil microorganisms' biodegradation activity being inhibited.

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INTRODUCTION

Natural biopolymers have several drawbacks, including quick biodegradability, low chemical reactivity, and low mechanical strength and miscibility. However, considering the ultimate application, there are numerous chances to improve these qualities. Amylose and amylopectin chains are the building blocks of starch (Jin *et al.* 2013; Zhong *et al.* 2013), and tuber crops provide a plentiful supply. However, its hydroscopic character, low mechanical strength, and high rate of natural biodegradation must be addressed before it can be used. Starch's tensile strength, hydrophilicity, and sensitivity to natural microbes have been improved by combining it with other natural and synthetic polymers such as lignin (Baumberger *et al.* 1998) and polypropylene (Pang *et al.* 2013).

Lepifre *et al.* (2004) found that combining lignin with starch improved the tensile properties of lignin-starch composites exposed to gamma rays. Irradiation improves lignin's molecular interactions with starch, which are mediated through radical scavenging behaviour. Lignin supplementation of up to 30% significantly reduces stress and elongation at break. In a separate study, larger lignin to starch ratios were found to improve the tensile strength of starch films (Çalgeris *et al.* 2012).

The hydrophobicity of lignin may also interfere with its tensile characteristics. As previously reported, lignin-polylactic acid blends with lignin loading of 0-1% against 5% showed the superior mixing and tensile qualities. This shows that lignin's bulky chemical structure disrupts molecular interactions in lignin-polylactic acid blends at high concentrations (Gordobil *et al.* 2014). Adding lignin (5 to 20%) and changing the thickness (0.27 to 1.03 mm) inhibits soil microorganism-caused biodegradability (Majeed *et al.* 2014, 2015b). In reality, the tensile qualities of any composites/blends play a critical role in determining performance and resistance over the time. There is very little information on the effect of lignin on urea cross-linked starch (UcS) tensile strength losses in soil (Majeed *et al.* 2015a).

Starch is hydrophilic and its losses through biodegradation are faster than those of lignin, which is hydrophobic in nature. Therefore, mixing of lignin with starch could tune the resistance of the later in natural environment. This way, rate of starch losses could be slowed down for optimized use in applications. Therefore, this study examined the tensile properties of lignin mixed UcS biocomposites as they degraded in a soil anaerobic microcosm. The influence of varying lignin loadings on biocomposite tensile characteristics was investigated.

EXPERIMENTAL

Materials

Tapioca starch (Kapal ABC brand, Ipoh, Malaysia) was purchased from a local store. Alkaline kraft lignin (Sigma Aldrich, St. Louis, USA) and di-sodium tetraborate heptahydrate (R&M Chemicals, India) were purchased, and granular urea 46% N (PETRONAS Chemicals Fertilizer Sdn Bhd, Kedah, Malaysia) was received as a sample.

Lignin Reinforced UcS

The method for creating biocomposites of lignin reinforced UcS films is described elsewhere (Majeed *et al.* 2016a; Majeed 2016b) and the process is systematically presented in Fig. 1. Briefly, 5 g of tapioca starch is solubilized for 30 min at 80 °C in distilled water in the presence of 2.0 g of urea and 0.45 g of di-sodium tetraborate heptahydrate. After that, different weight percentages (5, 10, 15, and 20 wt%) of lignin were added to obtain final biocomposites. Films were obtained after pouring into a plastic tray and drying at 45 °C.

The specimens were post-cured at 105 °C for 2 h. Biocomposites were named UcS0%L, UcS5%L, UcS10%L, UcS15%L, and UcS20%L based on the weight percent of lignin. The letters UcS and L stand for urea-crosslinked starch and lignin, respectively. The non-biodegraded composition of each biocomposites presented here is the same as in previous work (Majeed 2016b) and were employed as a reference to biodegraded compositions.

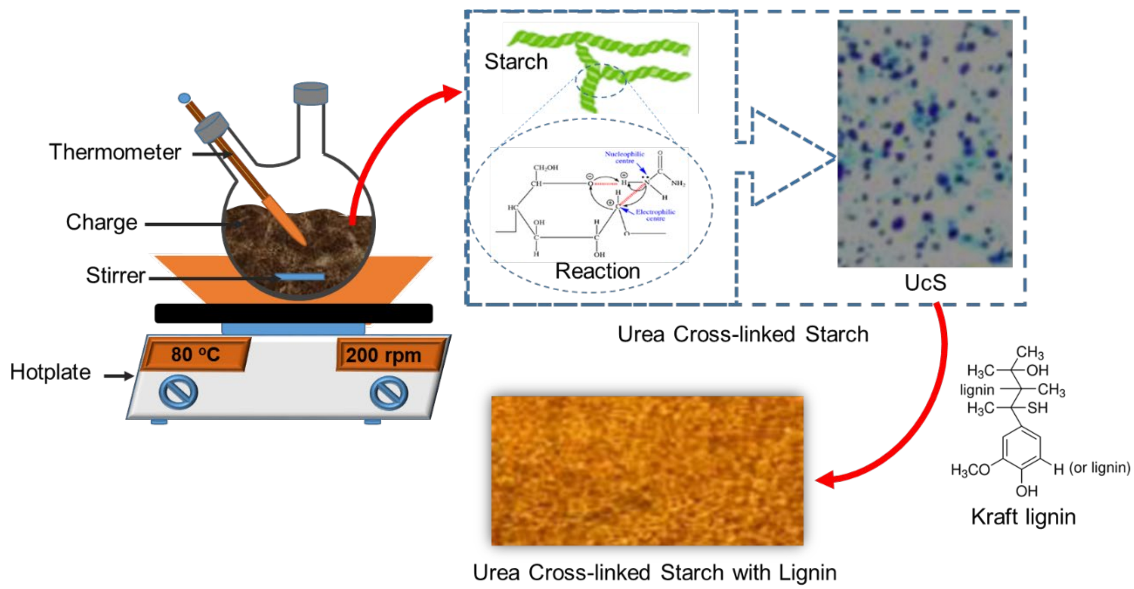

Fig. 1. Preparation of Urea-crosslinked biocomposites reinforced with lignin

Biodegradability Assay

The biocomposites were cut into 9 × 3 cm squares with a thickness of 0.54 ± 0.11 mm. A 9.5 × 6.5 cm muslin fabric was used to cover each sample prior to treatment with anaerobic soil microcosm, and it was then placed directly beneath anaerobic soil (Fig. 2). Soil used in this study was collected from Agriculture land, Perak, Malaysia. Soil sampling, physicochemical characteristics, and anaerobic microcosm preparation are detailed elsewhere (Majeed *et al.* 2018). With up to 7 days of exposure, maximum interaction between the biocomposite film and the anaerobic soil was ensured. In their natural form, biocomposite films are exceedingly soft.

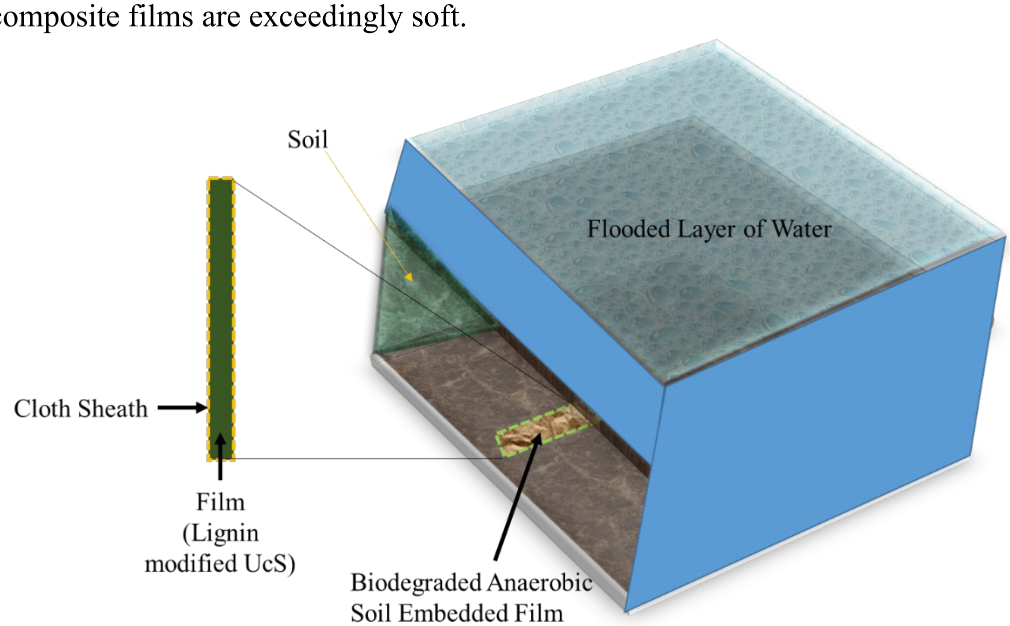

Fig. 2. Representation of experimental setup of anaerobic conditions applied to biodegradation lignin reinforced urea crosslinked starch biocomposites

After biodegradation, these films become increasingly brittle and fractured, and the experimental duration for valid samples to conduct tensile tests cannot be extended beyond 7 days. Biodegraded biocomposite films were collected and brushed clean to remove soil particles that had been deposited on the cloth and film. A desiccator was used to store and dry biocomposite films. Biocomposites were conditioned for 48 h at 28 °C and 70% relative humidity before tensile testing.

Tensile Properties Testing

Using a Testometric material testing machine (Instron, Wycombe, UK) with 10 kN force, biodegraded biocomposites were evaluated for tensile characteristics such as Young's modulus, elongation at break, and tensile strength. WinTestTM software was used to examine the machine data. Biocomposites were tested according to ASTM D638-14 (2014). Biocomposites were put to the test at a grip separation of 30.0 mm, a crosshead speed of 8.0 mm/min, and a force of 1 kN. Each biocomposition was tested at the same day in the same lab. The data for each biocomposites was averaged over five measurements. In addition, percent loss was calculated from the difference between pristine and biodegraded biocomposites' tensile properties. Maximum force was divided by the cross-sectional area of the specimen (width x thickness in mm) to obtain tensile strength (MPa) (Bourtoom and Chinnan 2008). WinTestTM analysis software was used to obtain the values of elongation at break (%) and Young's modulus (%).

Statistical Analysis

Origin Software was used for the statistical analysis (Version 8.5.1, OriginLab Corporation, Northampton, USA). Only data with a significance threshold of 0.05, a pair-sample t-test was used.

RESULTS AND DISCUSSION

Figure 3 depicts the effect of different lignin percentages on the tensile properties of pristine and biodegraded lignin reinforced UcS biocomposite films in an anaerobic soil microcosm. Lignin delayed the loss of tensile characteristics of biodegraded biocomposite films compared with control films, although an increase in lignin loadings of 5 to 20% is blamed for a higher rate of percent loss in elongation at break and tensile strength than Young's modulus.

The loss of tensile strength in biodegraded biocomposite films containing 15 to 20% lignin was substantially larger than in biodegraded control UcS0 percent L film, as illustrated in Fig. 2. Biocomposites with lignin addition had a lower loss in elongation at break, ranging from 34.14 to 51.22%, compared to controls, which had a loss of 66.73%. After biodegradation, the Young's modulus for lignin mixed UcS decreased from 31.03 to 37.59%, which was less than the 61.47% reported for biodegraded controlled UcS. The decrease in tensile strength (MPa) for biodegraded UcS was 11.02 to 21.57% for 5% and 10% lignin loading, which was lower than the 41.69% found for control. The tensile strength losses in biodegraded biocomposites were found to be larger at 15% and 20% than in control biocomposites. Water acted as a potent plasticizer against starch in biocomposites (Baumberger *et al.* 1998), and the same was observed in a water-saturated anaerobic soil microcosm.

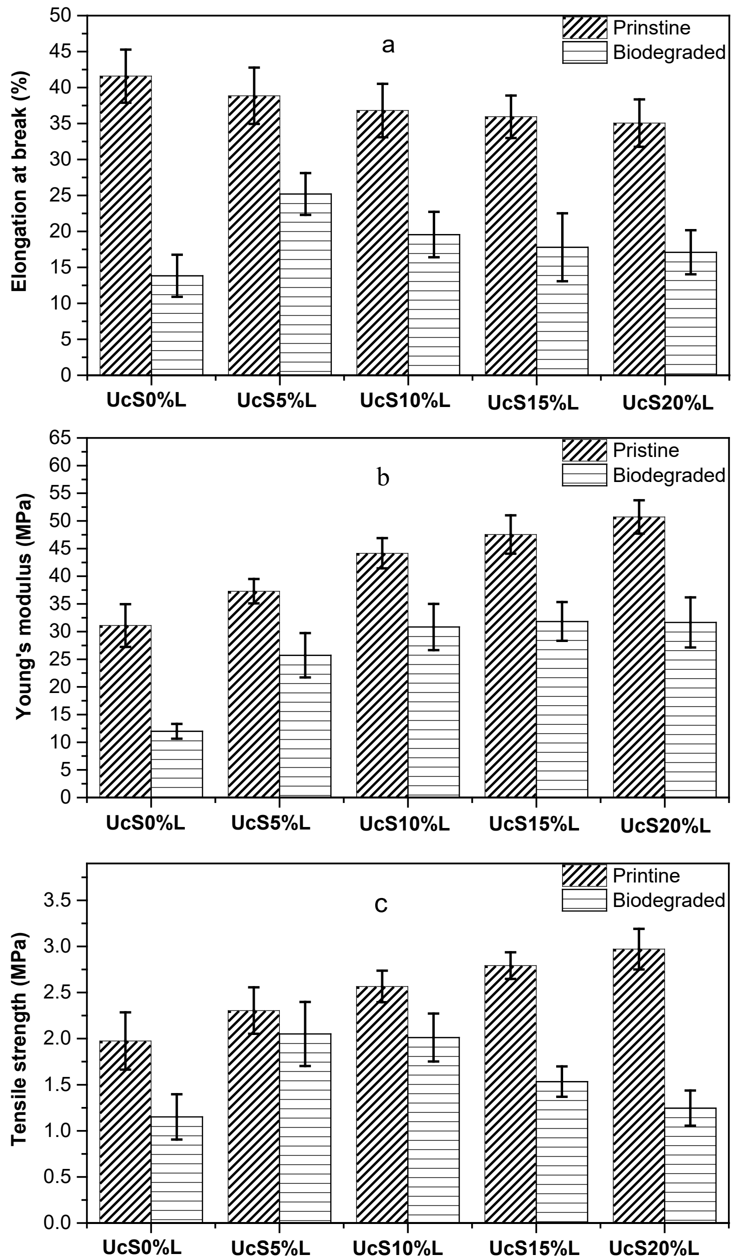

Fig. 3. Tensile characteristics of lignin-reinforced UcS composite films in anaerobic soil: (a) elongation at break, (b) Young's modulus, and (c) tensile strength

Virgin biocomposite films, upon contact with water, possibly strengthen the transient collision of molecules positioned at the interface of lignin and starch. Because of starch solubility and accessibility to soil microorganisms during their activity against films in an anaerobic environment, this alteration promotes higher starch losses. According to tensile experiments, lignin may have reduced the loss of tensile characteristics in biodegraded biocomposite films under water-saturated soil conditions.

Baumberger *et al.* (1998) reported that starch-lignin biocomposites involve disintegration in the structure of the starch due to increase its water solubility. The incorporation of kraft lignin into starch is known for poorer mechanical properties at 58% of water contents due to poor compatibility. But an increase in 58% to 71% of water content of the starch film may improve the tensile properties due to water's role in enhancing the biocompatibility of the hydrophilic starch with relatively high hydrophobicity of lignin in biocomposites. Therefore, anaerobic conditions maintained in water saturated soil explain that the water greatly contributes to the lignin through stabilizing the starch-rich phase of the biocomposites. This supports the data and trends shown in Fig. 3 and Table 1.

It is recommended that future work should focus on improving the interfacial interaction between hydrophobic lignin and hydrophilic starch in polymeric blends in order to further improve the mechanical strength and hence ultimately the resistance to natural losses.

Table 1. Tensile Test of Biodegraded Lignin Reinforced UcS Composite Films in Anaerobic Soil Condition

Biodegraded Films	Elongation at Break (%)		Young's Modulus (%)		Tensile Strength (MPa)	
	% Loss	*t*-test *p*-value	% Loss	*t*-test *p*-value	% Loss	*t*-test *p*-value
UcS0%L	66.73	0.000**	61.47	0.000**	41.69	0.009*
UcS5%L	34.14	0.000**	31.03	0.000**	11.02	0.652*
UcS10%L	46.86	0.000**	30.16	0.006**	21.57	0.030*
UcS15%L	50.48	0.000**	33.04	0.000**	45.06	0.000**
UcS20%L	51.22	0.000**	37.59	0.000**	58.04	0.000**

* Significant difference (p <0.05), ** Highly significant difference (p<0.001)

CONCLUSIONS

1. Higher lignin loadings in urea crosslinked starch (UcS) reduced the losses in elongation at break and Young's modulus, during biodegradation in anaerobic soil, according to the findings of this study.
2. At larger lignin loadings, the tensile strength showed some fluctuations, indicating that the effect on losses was greater than the control film without lignin.
3. The statistical test revealed that lignin treatment has a significant impact on the tensile properties of biodegradable UcS.
4. Lignin's role in UcS may improve performance in natural environments, and it should be used in all similar applications.
5. This work has the limitation that it studied the tensile losses in one type of soil microcosm. The work needs to be extended to a different set up of soil microcosm for comparative assessment of natural losses.

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