

Valorization of Soda Lignin from Wheat Straw Solid-State Fermentation: Production of Oleogels

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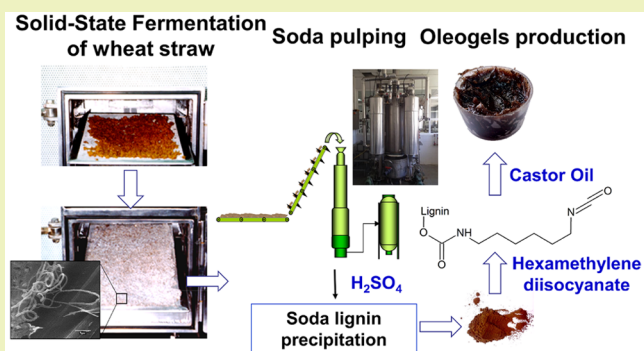
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Supporting Information

ABSTRACT: This work describes the solid-state fermentation (SSF) of wheat straw with *Streptomyces* sp. MDG147 and further soda-pulping process to obtain wheat straw soda lignins (WSLs). Subsequently, these WSLs were NCO-functionalized with 1,6-hexamethylene diisocyanate and then dispersed in castor oil to achieve stable oleogels. The WSLs were characterized using standard analytical methods, gel permeation chromatography, Fourier transform infrared spectroscopy, differential scanning calorimetry, and thermogravimetric analysis. Rheological properties of oleogels were determined by means of small-amplitude oscillatory shear and viscous flow measurements. The enzymatic profile and production of lignin–carbohydrate complexes were recorded along the growth time of *Streptomyces*, whose life cycle was achieved after 7 days. NCO-functionalized WSL was able to chemically interact with castor oil via urethane bonding, providing oleogels with suitable rheological characteristics. Linear viscoelastic functions and viscosity values of oleogels were higher when wheat straw was submitted to SSF using *Streptomyces*, turning out in stronger oleogels.

KEYWORDS: Actinobacteria, Castor oil, Diisocyanate, FTIR, Lubricating grease, Rheology, *Streptomyces*



INTRODUCTION

The growing interest in green and sustainable chemistry has contributed to calling attention to biomass and, specifically, to lignocellulosic feedstock. This resource constitutes a promising, renewable, and vast supply for chemicals, without competition with food applications compared to, e.g., starch or vegetable oils.¹ Among lignocellulosic biomass, wheat straw is one of the main agricultural residues with great potential because of its wide availability and low cost.^{2,3} Wheat straw is an abundant byproduct from wheat production in many countries and contains 35–45% cellulose, 20–30% hemicelluloses, and around 15% lignin.⁴ Lignin is an aromatic biopolymer traditionally considered as a waste product in several industrial processes such as pulp and paper mills and whose most widespread use is as fuel to self-recover part of the energy consumed during the process. Its storage or discharges into the environment cause serious pollution problems. However, the potential use of lignin to obtain high added-value products has not been deeply studied and is nowadays being considered in many research works.^{5–7}

On the other hand, as it is well-known, biological pretreatment is sometimes carried out before delignification

process (pulping stage) in order to reduce chemical or energy consumption during this process. In this sense, it has been demonstrated that actinobacteria such as *Streptomyces* are able to solubilize lignin from grass residues producing lignin–carbohydrate complexes, named APPL (acid-precipitable polymeric lignin), through the production of hemicellulolytic (xylanases, mannanases) and oxidative (laccases, peroxidases) enzymes.^{8–10} It is noteworthy the suitability of these Gram-positive filamentous bacteria for processes involving solubilization instead of mineralization of lignin as white-rot fungi preferentially do. However, the influence of this biological pretreatment on the residual lignin characteristics obtained after the pulping process has not been studied before, which may affect the further use of the mentioned lignin.

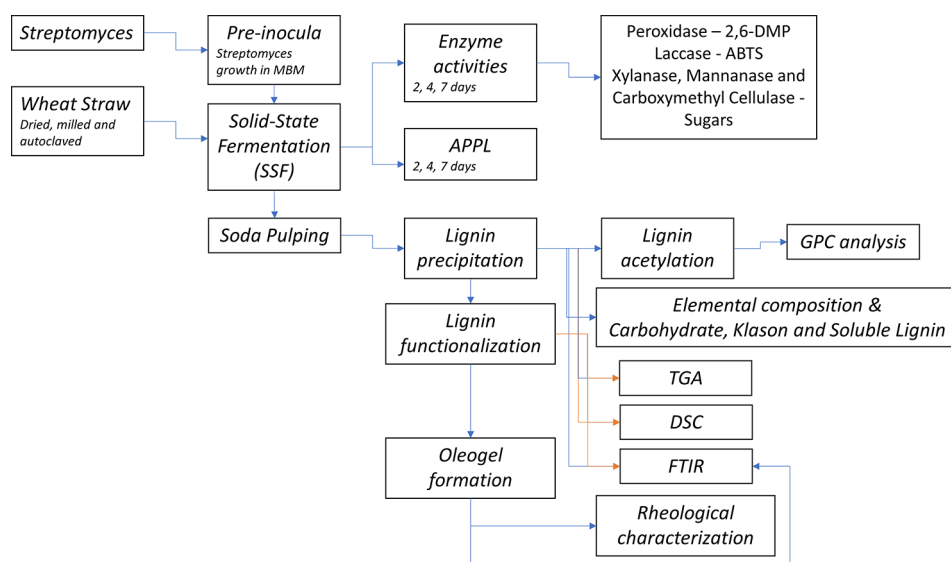
Different fossil fuels-based industries are facing difficulties associated with stronger politics against climate change and resources exhaustion. In this sense, the widespread use of fuels and of mineral oils in particular during the last century is now

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Scheme 1. Synopsis of Wheat Straw Treatments and Lignin Modifications To Produce Oleogels and Characterization Methodology Followed



being replaced by vegetable oils.^{11–14} Nevertheless, polyolefins, polyureas, and metal-based soaps are still the materials mostly used to gellify oily phases.^{15,16} In order to replace these traditional thickener agents, other substances derived from renewable resources such as cellulose,¹⁷ chitin and chitosan,¹⁸ or lignocellulosic materials¹⁹ have been evaluated. Moreover, isocyanate molecules have been extensively employed as chemical modifiers in a wide variety of biomass²⁰ and cross-linkers for gel formation.²¹ In a previous work, the 1,6-hexamethylene diisocyanate (HMDI) was found the most suitable modifier to impart proper gel-like rheological properties,²² which could suit, depending on diisocyanate concentration and lignin/diisocyanate ratio, a wide range of applications such as lubricant greases, coatings, sealants, or adhesives.^{12,19} Taking into account these considerations, here, different lignin byproducts were evaluated as potential thickeners in oil media, in order to obtain oleogels potentially applicable as biolubricating greases. In particular, the effect that SSF of wheat straw with *Streptomyces* sp. MDG147 exerts on the rheological properties of NCO-functionalized wheat straw soda lignin-based oleogels in castor oil was analyzed in this study.

MATERIALS AND METHODS

Materials. Castor oil (Laboratorios Guinama, Valencia, Spain), with 82.5, 7.0, and 5.3% content of ricinoleic, linoleic, and oleic acids, respectively, was used as the base oil without any purification. Pure grade HMDI ($\geq 98.0\%$) from Sigma-Aldrich, was selected to functionalize WSL. All other common reagents and solvents employed were of analytical grade, also purchased from Sigma-Aldrich.

Methods. For a better understanding and tracking of the whole process, Scheme 1 shows a flowchart which displays the sequence of wheat straw treatments and lignin modifications to produce oleogels as well as the characterization protocols followed.

Streptomyces Strain and Growth Conditions. *Streptomyces* sp. MDG147 was isolated from lignocellulosic residues and identified based on the information reported by Williams et al.²³ This strain was deposited in the culture collection of the MICRODEG Research group of the University of Alcalá (Universidad de Alcalá de Henares, Madrid, Spain). Spores suspensions were kept at $-20\text{ }^{\circ}\text{C}$ in 20% (w/v) glycerol until use, and for experiments, microorganisms were routinely

cultured up to sporulation in GAE (glucose–asparagine–yeast extract)²⁴ or soy–manitol²⁵ agar dishes.

Solid-State Fermentation of Wheat Straw. Wheat straw (*Triticum aestivum* var. *maestro*) was ground in a Janke and Kunkel A-10 mill to pass through a 40-mesh screen, air-dried at $50\text{ }^{\circ}\text{C}$, and autoclaved at $121\text{ }^{\circ}\text{C}$ for 20 min. The substrate was inoculated under SSF conditions with preinocula obtained as follows: standardized spore suspensions (10^7 cfu mL⁻¹) from the *Streptomyces* sp. MDG147 strain were inoculated into flasks containing MBM²⁶ supplemented with 0.6% (w/v) yeast extract and maintained under shaking conditions overnight at $28\text{ }^{\circ}\text{C}$.

Then, flasks (500 mL) containing 10 g of wheat straw were inoculated with 56 mL of preinoculum to obtain 85% moisture and were incubated under SSF at $28\text{ }^{\circ}\text{C}$ for 2, 4, and 7 days, prepared by triplicates. Scale-up experiments were carried out in steel tray bioreactors with five trays ($38 \times 28\text{ cm}^2$) each. A 1 kg amount of wheat straw was inoculated with 560 mL of preinoculum and distributed at 100 g/tray. Bioreactors were maintained at $28\text{ }^{\circ}\text{C}$ for 7 days and periodically aerated with filtered air ($0.22\text{ }\mu\text{m}$ membrane). Uninoculated controls were incubated under the same conditions.

APPL and Alkali-Lignin Extraction. After 2, 4, and 7 days of incubation in SSF conditions, APPL was extracted from wheat straw transformed by the *Streptomyces* strain.²⁷ To obtain APPL, 20 mL of distilled water/g was added to the transformed wheat straw and steamed at $100\text{ }^{\circ}\text{C}$ for 1 h, then filtered through Whatman no. 54 filter paper, and washed again with 100 mL of water at $80\text{ }^{\circ}\text{C}$. The APPL was precipitated from the supernatants by acidification to pH 1–2 with 12 M HCl. Precipitates were collected by centrifugation (12000 g, 10 min), washed twice with deionized acidic water, freeze-dried in a Christ Alpha 1-4 freeze-dryer with LDC-1 M controller, and weighed. For alkali-lignin extraction the same procedure was followed but lignin solubilization was carried out with 0.1 M NaOH.

Enzyme Assays. Transformed wheat straw (10 g) was extracted with 15 mL of distilled water after 2, 4, and 7 days' growth. The mixtures were sonicated for 15 min and then filtered through Whatman no. 1 filter paper to obtain the crude enzyme extract²⁸ in which peroxidase, laccase, xylanase, mannanase, and carboxymethyl cellulase enzymatic activities were measured (see detailed protocols in the Supporting Information). All enzyme assays were made in triplicate.

Soda Pulping. Fermented and uninoculated wheat straw were subjected to soda-pulping process into a 20 L rotary pressurized vessel with a jacket-type electrical heater controlled by a computer to set the cooking temperature. Cooking conditions were 9:1 liquor/straw ratio, 15% odw (oven dry weight) NaOH, $160\text{ }^{\circ}\text{C}$ cooking temperature and

were maintained for 90 min at maximum temperature. The resulting soda pulps were filtered, and the black liquors were collected. Then the lignin was precipitated following a protocol described elsewhere (see detailed information in the Supporting Information).

Preparation of Oleogels. Both WSLs were modified using HMDI, in 1:2 lignin/HMDI weight ratio, as described in previous studies,²² and afterward these NCO-functionalized lignin samples (FWSLs) were used to prepare oleogels following the protocol described elsewhere,²² selecting 25% FWSL concentration.

Characterization of WSLs, FWSLs, and Oleogels. The compositions of soda lignins were determined by standard analytical methods (National Renewable Energy Laboratory NREL/TP-510-42618). The samples were subjected to quantitative acid hydrolysis in two steps to determine the carbohydrate composition. The hydrolyzed liquids obtained were then analyzed for sugar contents using an Agilent Technologies 1260 HPLC fitted with a refractive index detector and an Agilent Hi-Plex Pb column operated at 70 °C with Milli-Q water as mobile phase pumped at a flow rate of 0.6 mL min⁻¹. The solid residues remaining after the acid hydrolysis were considered acid insoluble lignin (Klason lignin). Ash was determined following the standard UNE 57050:2003.

Wheat straw lignin (WSL) molecular weight data and glass transition temperatures for WSL and FWSL samples were obtained from gel permeation chromatography (GPC) and differential scanning calorimetry (DSC) measurements, respectively, as detailed in the Supporting Information.

Elemental analyses were carried out following the UNE-EN 15104:2011 standard, using a Thermo Finnigan elemental analyzer, model Flash EA 1112.

For the two-dimensional heteronuclear single quantum coherence (2D HSQC NMR) spectra, a Bruker Avance III 500 MHz (Bruker, USA) spectrometer was used.

Fourier transform infrared spectra (FTIR) were carried out using a JASCO FT/IR-4200 apparatus. The spectra were obtained in the transmission mode, at 4 cm⁻¹ resolution, in a wavenumber range of 400–4000 cm⁻¹.

Thermogravimetric analysis (TGA) measurements were taken from room temperature to 600 °C at 10 °C min⁻¹, using a SII TG/DTA 6200 apparatus, under N₂ inert atmosphere.

Rheological characterization of FWSLs-based oleogels was carried out with an ARES (Rheometric Scientific) controlled-strain rheometer, using roughened plate–plate (25 mm diameter, 1 mm gap) geometry to prevent wall-slip.²⁹ Small-amplitude oscillatory shear (SAOS) tests were performed inside the linear viscoelastic region in the frequency range of 0.03–100 rad/s and viscous flow tests in a shear rate range of 0.01–100 s⁻¹, at 25 °C. Measurements were made by replicate 1 week after oleogel preparation.

RESULTS AND DISCUSSION

Monitoring Wheat Straw Fermentation: APPL Obtaining and Enzyme Determinations. As shown in Figure 1, highest xylanase and mannanase activities were detected after 4 days of incubation and were coincident with the highest degree of solubilization of lignocellulose (estimated as APPL). Although a synergistic action of both enzymes could be considered as responsible for this solubilization, it is important to consider that the ratio arabinoxylan/glucomannane in nonwoody angiosperm is 20:1.³⁰ For this reason, the main role for APPL solubilization should be attributed just to the production of xylanase activity. In a previous work, once APPL was extracted, the residual wheat straw fermented with this strain was characterized by analytical pyrolysis associated with gas chromatography–mass spectrometry (Py-GC/MS). In the residual wheat straw, a decrease in the relative amount of the xylan marker 2,3-dihydro-5-methylfuran-2-one was detected after 2 days of incubation and attributed to the activity of xylanases produced by the microorganism at the beginning of

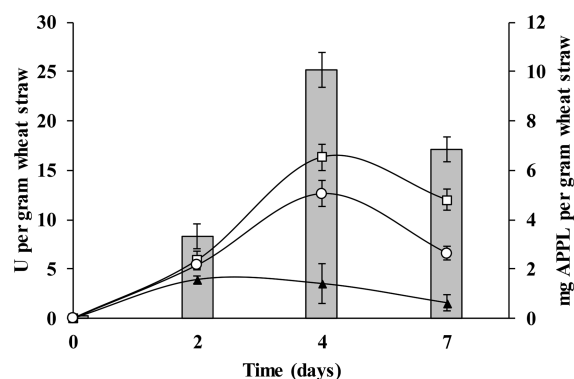


Figure 1. Production of extracellular xylanase (□), mannanase (○) and CMCase (▲) activities and APPL (bars) during the growth of *Streptomyces* sp. MDG147 in solid-state fermentation on wheat straw. All values are the mean of three determinations ± SD.

the incubation time.³¹ In this figure, an important decrease in the APPL amount can also be observed after 7 days incubation. The previous chemical characterization of this polymer by Py-GC/MS showed a lignin/carbohydrate ratio of 80:20.³¹ In addition, the molar proportion of the phenylpropane-type moieties in this lignin shows that G-type units (3-methoxy-4-hydroxy-phenylpropane) are predominant over the H (4-hydroxy-phenylpropane) and S (3,5-dimethoxy-4-hydroxy-phenylpropane) units (ratio H/G/S, 18:62:20), which confers this lignin a high degree of condensation being difficult to be degraded. According to this, the APPL decrease observed could be mainly attributed to the carbohydrate moiety degradation by the microorganism once the lignin–carbohydrate complexes (APPL) were solubilized from wheat straw. Finally, as shown in Figure 1, the CMCase activity does not seem to have a relevant role in the APPL production.

In order to improve the yield of solubilized lignin from wheat straw by the microorganism, an alkaline extraction with 0.1 M NaOH was carried out. In Figure 2, the alkali-lignin extracted

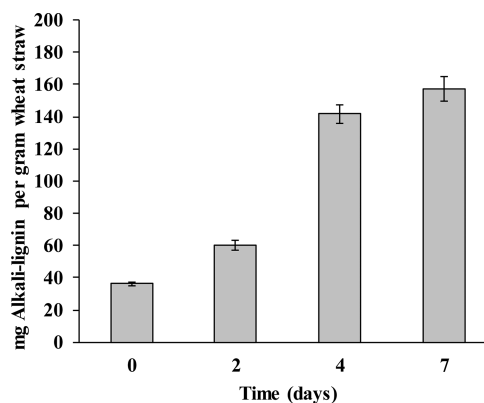


Figure 2. Alkali lignin obtained from wheat straw fermented with *Streptomyces* sp. MDG147 along the time course of growth. All values are the mean of three determinations ± SD.

along the time course of *Streptomyces* growth is shown. As expected, the amount of lignin solubilized from wheat straw using alkali was higher than when using water, reaching a maximum solubilization of 160 mg/g wheat straw after 7 days incubation. Taking into account that alkali lignin obtained after 4 days is 14-fold compared with APPL and 25-fold after 7 days,

it was decided to incubate this microorganism for 7 days in the scale-up process.

Alkali-Lignin Production in Tray Bioreactors. After 7 days of incubation a complete life cycle was achieved by the microorganism as can be inferred from the sporulation degree reached in the fermentor. The resulting material was used for soda pulping and further functionalization.

Composition and Molecular Weight of soda WSLs.

Table 1 shows the composition of both lignins (control and Fe) isolated from the black liquor resulting after soda pulping. The yield of Klason lignin is very similar in control and fermented samples (60.6% and 60.1%, respectively). As it is known, the amount of Klason lignin (insoluble lignin) is related to the purity of the lignin.³² Thus, the method used here is considered adequate to obtain an acceptable purity of this biopolymer. Other authors^{32,33} have also isolated lignin from black liquor after soda pulping of olive tree pruning with different purities (4.6% and 84.0%, respectively). However, although the method used by these authors for lignin precipitation was the same as that applied in this work, the raw material used was different and the soda cooking was performed under different conditions. Moreover, in another publication³³ lignin was washed with deionized water in order to remove salts, which can be formed during lignin precipitation process.³⁴ The lack of this stage could explain the high ash content of our samples as well as in other data reported elsewhere.³²

As can be also observed in Table 1, the amount of soluble lignin in the fermented sample was significantly lower in the control. This decrease is probably due to the action of the *Streptomyces* during SSF. During this biopretreatment, actinobacteria facilitate the delignification of the initial biomass, favoring the removal of the low molecular weight degradation products and hydrophilic derivatives of lignin, both components of soluble lignin.

On the other hand, both lignin samples show similar carbohydrate profiles due to the LCCs (lignin-carbohydrate complexes) present in the initial raw material. As other authors have also reported,³² the predominant elemental sugar in both samples was xylose. The reason behind this could be the well-known peeling reaction which consists of a successive depolymerization of polysaccharides starting from the reducing end group, which affects mainly to hemicelluloses such as xylans. Moreover, the control sample presented a higher xylan content suggesting that *Streptomyces* could partially remove this kind of carbohydrate during the pretreatment and/or facilitate its removal during the cooking process.

Table 1 also shows results of the elemental analysis of WSLs. This analysis demonstrated higher concentrations of C, H, and N in the fermented sample, as a consequence of the soluble lignin removal and a probable increase in proteins concentration. Moreover, it is known that oxidative enzymes in *Streptomyces* attack the β -O-4 bonds, generating new OH groups^{31,35} available for subsequent functionalization reactions with NCO groups. The semiquantitative 2D HSQC NMR spectra of both control and Fe samples (see Figure S2 in the Supporting Information) confirmed a reduction of around 11% in β -O-4 linkages for the Fe WSL.

On the other hand, to elucidate the biological action of the *Streptomyces* on the molecular weight distribution of WSLs, GPC analyses were performed. The number-average molecular weight (M_n), the weight-average molecular weight (M_w), and polydispersity index (M_w/M_n) were calculated and depicted in Table 1. Both lignin samples studied show very similar average

Table 1. WSL Composition and Molecular Weight Data

sample	code	Klason lignin (%)	soluble lignin (%)	glucose (%)	xylose (%)	galactose (%)	arabinose (%)	mannose (%)	ash (%)	% C	% H	% N	M_n	M_w	M_w/M_n
control	control	60.6 ± 1.0	9.7 ± 0.2	2.1 ± 0.2	7.9 ± 0.1	0.0 ± 0.0	1.8 ± 0.0	0.0 ± 0.0	14.9 ± 0.6	47.14	5.60	2.30	3466	7485	1.84
	Fe	60.1 ± 1.4	6.0 ± 0.1	1.8 ± 0.1	6.2 ± 0.1	0.0 ± 0.0	1.3 ± 0.0	0.0 ± 0.0	12.3 ± 0.8	52.01	6.11	2.85	3308	6830	1.82

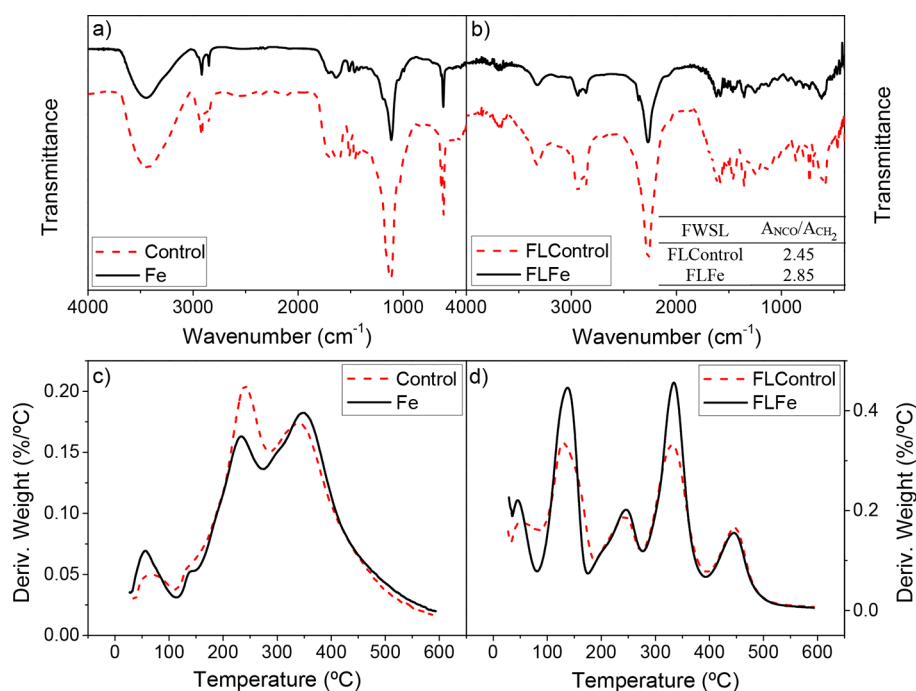


Figure 3. FTIR spectra of (a) WSLs and (b) FWSLs and thermal degradation curves, under inert atmosphere, for (c) WSLs and (d) FWSLs.

Table 2. TGA Characteristic Parameters for WSLs and FWSLs

sample	code	T_{onset} (°C)	T_{max} (°C)	T_{final} (°C)	ΔW (%)	residue (%)
control	Control	44/127/205/312	68/137/241/342	87/155/261/408	3/21/26	49
fermented	Fe	40/129/189/321	56/137/233/350	77/145/251/411	4/17/29	50
FL control	FLControl	34/102/209/302/424	36/126/242/325/446	54/150/258/364/478	9.5/24/13/25/12	16.5
FL fermented	FLFe	33/108/206/310/425	48/130/244/336/449	64/161/266/360/475	8/25/15/27/11	14

molecular weights and polydispersity values. The slightly higher molecular weight found in the control sample is in agreement with the soluble lignin removal caused by the action of the actinobacteria used during SSF. Besides, average molecular weights of these studied lignins are higher than those previously reported elsewhere, despite polydispersity being quite similar.³⁶

FTIR and Thermal Characterization of WSLs and FWSLs. Infrared spectra were used to characterize the main functional groups present in the samples studied.^{37–39} Panels a and b of Figure 3 show WSLs (control and Fe) and FWSLs spectra, respectively. First of all, a wide peak centered at around 3440 cm^{-1} , corresponding to O–H stretching absorption in phenolic and aliphatic structures, is displayed in both nonmodified WSLs, as well as bands at 2920 and 2845 cm^{-1} , related to the C–H asymmetric and symmetric stretching in aromatic methoxyl groups and methyl and methylene groups of side chains, respectively (Figure 3a). These bands are less intense for Fe than control lignin, suggesting a decrease in the number of aliphatic methylene and methyl groups during SSF. Also, a peak corresponding to the C=O stretching in the unconjugated ketones, carbonyl, and ester groups was observed at 1710 cm^{-1} . The intensity of this peak is reduced in the Fe sample, probably as a consequence of some hemicellulose consumption by the *Streptomyces* action, as mentioned above. Moreover, the spectra show the typical lignin bands at 1610 and 1516 cm^{-1} due to aromatic skeleton vibrations and 1463 cm^{-1} corresponding to asymmetric bending deformation of methyl and methylene groups. On the other hand, it is apparent that WSL samples belong to the HGS type, with bands at 1327 ,

1257 , and 823 cm^{-1} , attributed to C–O bonds in syringyl and guaiacyl rings and C–H bonds out-of-plane in positions 2 and 6 of the S units and in all positions of the H units, respectively, as well as the band at 1160 cm^{-1} , typical of conjugated C=O bonds in ester groups of HGS lignin. These bands appear as shoulders of the high intensity band located at 1105 cm^{-1} related to the asymmetric stretching of SO_4 groups, which appears as a result of the formation of salts during lignin precipitation.⁴⁰ The band around 1257 cm^{-1} slightly decreased after the fermentation treatment, as a consequence of the unlocking of lignin cross-links through lignin subunits removal.

Two new bands at 3330 cm^{-1} (N–H stretching vibrations) and 1570 cm^{-1} (N–H in-plane bending vibration) were observed once OH groups of WSLs reacted with isocyanate groups in the functionalization reaction with HMDI, thus leading to urethane linkages (Figure 3b). Afterward, the two peaks related with asymmetric and symmetric C–H stretching vibration of aliphatic methylene groups are clearly risen in FWSLs because of the methylene groups added by HMDI. However, the most noticeable change in FTIR spectra after functionalization is clearly the wide band appearing at 2270 cm^{-1} , corresponding to -NCO asymmetrical stretching vibration of free isocyanates groups. Comparison of the intensity of this band for both FWSLs, relative to the intensity of band corresponding to methylene groups, demonstrates that the number of free isocyanates sites is slightly higher for Fe sample, despite the fact of the more-attainable soluble lignin liberated because of the *Streptomyces* activity.⁴¹

Lignin glass transition temperature (T_g) is an important parameter for industrial lignin applications and processing.^{42,43} All the WSLs studied showed a T_g value around 42 °C. However, after NCO functionalization, this glass transition temperature increased to 92 °C for the Control and 96 °C for Fe.

TGA measurements were used to determine the thermal stability of the lignin samples (Figure 3c,d and Table 2).³⁹ For better understanding and comparison, the onset temperature (T_{onset}), temperature for the maximum weight loss rate (T_{max}), final degradation temperature (T_{final}), and weight loss (ΔW) were estimated for each thermal event during the analysis, as well as the final residue at the end of the experiment. As can be seen in Figure 3c, the SSF of wheat straw with actinobacteria and further soda pulping produce some changes in the lignin thermal degradation pattern. Paying attention to the derivative weight loss, after the first loss of moisture before 100 °C observed for the two lignins studied, a second wide-shoulder peak with T_{max} at 137 °C was also shown for both of them. This degradation stage is related to the hydroxyl groups dehydration.⁴⁴ The following thermal degradation stage, with T_{max} at around 231–241 °C, is a consequence of β -O-4 linkage breakdown and aliphatic side chains cleavage, while the one shown around 342–350 °C corresponds to disruption of C–C bonds among monolignols.⁴⁴ Some slight differences can be appreciated in these two peaks since the Control sample exhibits higher weight loss in the first one and Fe sample in the second one, as expected by the β -O-4 bonds disruption, which is also in concordance with lignin composition and the higher C concentration previously discussed (Table 1). This thermal behavior demonstrates that SSF with actinobacteria generates new bonds in lignin.

On the other hand, Figure 3d shows TGA curves for FWLSs, once functionalization was accomplished. Apart from the initial residual toluene loss under 100 °C, FWLSs thermograms also exhibit differences between both lignins analyzed. The peak centered around 126–139 °C, related to free isocyanate loss, is similar for both of them, but slightly higher for FLFe. The following two peaks are related to the two main peaks previously discussed for WSLs. Moreover, Figure 3d also displays that FWLSs show a new peak at around 445–450 °C, as also shown in previous work,^{20,22} which is a consequence of highly cross-linked structures promoted by diisocyanate reactions.

Characterization of FWLSs-Based Oleogels. FTIR spectra of oleogels (Figure S1 in the Supporting Information) were, all of them, almost identical, and similar to those previously reported for other NCO-functionalized commercial alkali-lignin-based oleogels.²² They present the typical O–H, N–H, and C=O bands, but the most interesting feature is the significant reduction in the band corresponding to free isocyanate moieties, which also diminished with time due to an internal curing process. This evolution affects both appearance and rheological properties of oleogel, deeply related to strengthening of the internal chemical structure.²² Monitoring of this loss was carried out by following the relationship between the areas of diisocyanate and methylene bands, respectively. This evolution is shown in Figure 4 for the two samples studied. Once the curing process was completed, i.e., total loss of free isocyanates, no longer were changes observed in oleogel properties.

Figure 5a depicts the mechanical spectra of FWLSs-based oleogels as a function of lignin type. As can be observed, the

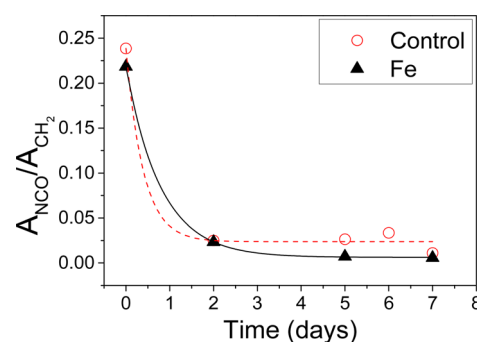


Figure 4. Evolution of isocyanate content with curing process for oleogels prepared with FWLSs.

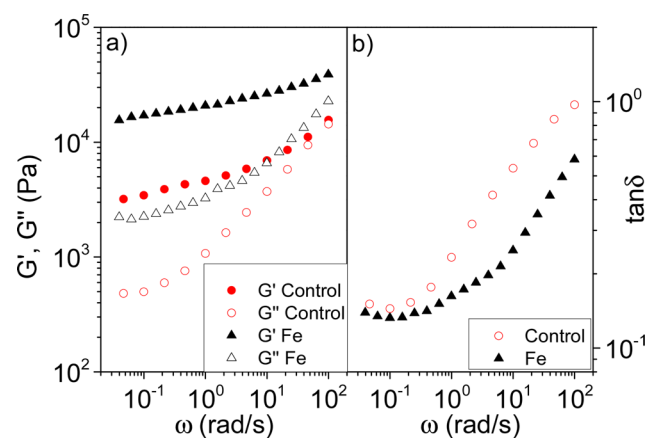


Figure 5. Frequency dependence of (a) the storage, G' , and loss, G'' , moduli and (b) the loss tangent for FWLSs-based oleogels.

linear viscoelasticity response is qualitatively similar for both oleogels studied. The values of storage modulus, G' , are generally higher than those found for the loss modulus, G'' , in the whole frequency range studied, with relatively low slope of the G' versus frequency plot and a tendency to reach a crossover between both SAOS functions at high frequencies. However, linear viscoelastic function values clearly increase when wheat straw was processed under SSF conditions using *Streptomyces* sp. MDG147 yielding higher values of G' and G'' , resulting in a linear viscoelastic response qualitatively and quantitatively similar to that found in traditional NLGI grades 1 and 2 lithium soap-based lubricating greases.^{15,45} The differences obtained may be related to both the higher C, H, and N contents in Fe sample, yielding more accessible aliphatic moieties, and the reduction of soluble lignin, which probably increases OH units because of the β -O-4 breakdown. This results in a more highly entangled structure, which guides to a higher oil confinement, higher NCO–lignin complex availability for remaining reaction with castor oil, and tougher arrangement because of the increase in H-bonds that can be obtained together with covalent bonds. The evolution of SAOS functions with frequency is typically found in highly entangled polymeric systems.⁴⁶ The relative elasticity of oleogels is almost independent of the type of WSL at low frequencies, as shown in Figure 5b, where the values of the loss tangent ($\tan \delta = G''/G'$) are plotted versus frequency. However, FLFe-based oleogel shows lower values of the loss tangent at high frequencies, indicating a higher relative elasticity.

Finally, Figure 6 shows the viscous flow behavior exhibited by the two oleogels. As can be seen, a shear-thinning behavior is

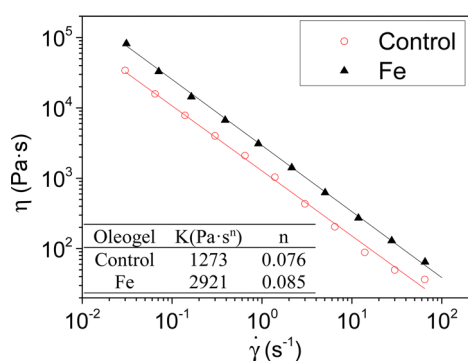


Figure 6. Viscous flow curves for FWLS-based oleogels.

apparent in both cases, which can be fitted fairly well to the power-law model:

$$\eta = k\dot{\gamma}^{n-1} \quad (1)$$

where k and n are the consistency and flow indexes, respectively. The viscosity values of FLFe-based oleogel are higher than those obtained with the control sample, resulting in a higher consistency index, despite the similar low values of the flow index found in both systems, typical of yielding materials as lubricating greases.^{15,45}

CONCLUSIONS

The enzymatic profile and the production of APPL were established along the time course of growth of *Streptomyces* sp. MDG147 on wheat straw under solid-state fermentation (SSF). The complete life cycle was achieved by the microorganism after 7 days of incubation as can be inferred from the sporulation degree reached in the fermentor. This SSF enhances the further soda pulping. The amount of Klason lignin is very similar in both the control and the fermented samples. However, the soluble lignin found in the fermented sample was lower than that of the control because of the action of the *Streptomyces* during SSF treatment, thus favoring the removal of low molecular weight degradation products and hydrophilic derivatives of lignin. The control sample presented a higher xylan content, suggesting that *Streptomyces* promotes the removal of this kind of carbohydrate during the pretreatment or, at least, eases this removal during the cooking process. WSLs chemical modification with HMDI allows a proper dispersion in castor oil and formation of chemical oleogels, via urethane bonding, with suitable rheological properties. The rheological response of these oleogels is affected by WSL type. Thus, linear viscoelastic functions and viscosity values clearly increase when wheat straw was submitted to SSF, showing values similar to those obtained with traditional lithium soap-based lubricating greases. This improvement has been related to a reduction in soluble lignin because of β -O-4 bonds disruption, together with an increase in C, H, and N content. In this work it was demonstrated for the first time the suitability of *Streptomyces* to obtain lignins from agricultural residues, which offer structural facilities to be applied for oleogels elaboration and their further use for sustainable lubricants production.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.7b04846.

Extended information on the experimental section, FTIR spectra, and 2D-HSQC spectra (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Laurichesse, S.; Avérous, L. Chemical modification of lignins: Towards biobased polymers. *Prog. Polym. Sci.* **2014**, *39*, 1266–1290.
- (2) Sarkar, N.; Ghosh, S. K.; Bannerjee, S.; Aikat, K. Bioethanol production from agricultural wastes: An overview. *Renewable Energy* **2012**, *37*, 19–27.
- (3) Kim, S.; Dale, B. E. Global potential bioethanol production from wasted crops and crop residues. *Biomass Bioenergy* **2004**, *26*, 361–375.
- (4) Del Río, J. C.; Rencoret, J.; Prinsen, P.; Martínez, A. T.; Ralph, J.; Gutiérrez, A. Structural characterization of wheat straw lignin as revealed by analytical pyrolysis, 2D-NMR, and reductive cleavage methods. *J. Agric. Food Chem.* **2012**, *60*, 5922–5935.
- (5) Nacas, A. M.; Ito, N. M.; De Sousa, R. R., Jr.; Spinacé, M. A.; Dos Santos, D. J. Effects of NCO:OH ratio on the mechanical properties and chemical structure of Kraft lignin-based polyurethane adhesive. *J. Adhes.* **2017**, *93*, 18–29.
- (6) Norgren, M.; Edlund, H. Lignin: Recent advances and emerging applications. *Curr. Opin. Colloid Interface Sci.* **2014**, *19*, 409–416.
- (7) Cortés-Triviño, E.; Valencia, C.; Franco, J. M. Influence of epoxidation conditions on the rheological properties of gel-like dispersions of epoxidized kraft lignin in castor oil. *Holzforchung* **2017**, *71* (10), 777–784.
- (8) Antonopoulos, V. T.; Hernández, M.; Arias, M. E.; Mavrakos, E.; Ball, A. S. The use of extracellular enzymes from *Streptomyces albus* ATCC 3005 for the bleaching of eucalyptus kraft pulp. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 92–97.
- (9) Moya, R.; Hernández, M.; García-Martín, A. B.; Ball, A. S.; Arias, M. E. Contributions to a better comprehension of redox-mediated decolouration and detoxification of azo dyes by a laccase produced by *Streptomyces cyaneus* CECT 3335. *Bioresour. Technol.* **2010**, *101*, 2224–2229.
- (10) Arias, M. E.; Blázquez, A.; Hernández, M.; Rodríguez, J.; Ball, A. S.; Jiménez-Morillo, N. T.; González-Vila, F. J.; González-Pérez, J. A. Role of a thermostable laccase produced by *Streptomyces ipomoeae* in the degradation of wheat straw lignin in solid state fermentation. *J. Anal. Appl. Pyrolysis* **2016**, *122*, 202–208.
- (11) García-Zapateiro, L. A.; Valencia, C.; Franco, J. M. Formulation of lubricating greases from renewable basestocks and thickener agents: A rheological approach. *Ind. Crops Prod.* **2014**, *54*, 115–121.

- (12) Tenorio-Alfonso, A.; Sánchez, M. C.; Franco, J. M. Preparation, characterization and mechanical properties of bio-based polyurethane adhesives from isocyanate-functionalized cellulose acetate and castor oil for bonding wood. *Polymers* **2017**, *9*, 132.
- (13) Sajjadi, B.; Raman, A. A. A.; Arandiyani, H. A comprehensive review on properties of edible and non-edible vegetable oil-based biodiesel: Composition, specifications and prediction models. *Renewable Sustainable Energy Rev.* **2016**, *63*, 62–92.
- (14) Li, Y.; Chou, S.-H.; Qian, W.; Sung, J.; Chang, S. I.; Sun, X. S. Optimization of soybean oil based pressure-sensitive adhesives using a full factorial design. *J. Am. Oil Chem. Soc.* **2017**, *94* (5), 713–721.
- (15) Martín-Alfonso, J. E.; Valencia, C.; Sánchez, R.; Franco, J. M.; Gallegos, C. Evaluation of different polyolefins as rheology modifier additives in lubricating grease formulations. *Mater. Chem. Phys.* **2011**, *128*, 530–538.
- (16) Paszkowski, M. Effect of grease thickener and surface material on rheological properties of boundary layer. *Colloids Surf., A* **2015**, *480*, 462–467.
- (17) Sánchez, R.; Stringari, G. B.; Franco, J. M.; Valencia, C.; Gallegos, C. Use of chitin, chitosan and acylated derivatives as thickener agents of vegetable oils for bio-lubricant applications. *Carbohydr. Polym.* **2011**, *85*, 705–714.
- (18) Lucio, B.; De La Fuente, J. L.; Cerrada, M. L. Characterization of phase structures of novel metallo-polyurethanes. *Macromol. Chem. Phys.* **2015**, *216* (20), 2048–2060.
- (19) Suhas, D. P.; Jeong, H. M.; Aminabhavi, T. M.; Raghu, A. V. Preparation and characterization of novel polyurethanes containing 4,4'-{oxy-1,4-diphenyl bis(nitromethylidene)}diphenol schiff base diol. *Polym. Eng. Sci.* **2014**, *54* (1), 24–32.
- (20) Gallego, R.; Arteaga, J. F.; Valencia, C.; Díaz, M. J.; Franco, J. M. Gel-like dispersions of HMDI-cross-linked lignocellulosic materials in castor oil: toward completely renewable lubricating grease formulations. *ACS Sustainable Chem. Eng.* **2015**, *3*, 2130–2141.
- (21) Gallego, R.; Arteaga, J. F.; Valencia, C.; Franco, J. M. Thickening properties of several NCO-functionalized cellulose derivatives in castor oil. *Chem. Eng. Sci.* **2015**, *134*, 260–268.
- (22) Borrero-López, A. M.; Valencia, C.; Franco, J. M. Rheology of lignin-based chemical oleogels prepared using diisocyanate cross-linkers: effect of the diisocyanate and curing kinetics. *Eur. Polym. J.* **2017**, *89*, 311–323.
- (23) Williams, S. T.; et al. Streptomyces and related genera. In *Bergey's Manual of Systematic Bacteriology*, Vol. 4; Williams, S. T., Sharpe, M. E., Holt, J. G., Eds.; Williams and Wilkins: Baltimore, MD, USA, 1989.
- (24) Hernández, M.; Rodríguez, J.; Soliveri, J.; Copa, J. L.; Pérez, M. I.; Arias, M. E. Paper-mill effluent decolorization by 50 *Streptomyces* strains. *Appl. Environ. Microbiol.* **1994**, *60*, 3909–3913.
- (25) Hobbs, G.; Frazer, C. M.; Gardner, D. C. J.; Cullum, J. A.; Oliver, S. G. Dispersed growth of *Streptomyces* in liquid culture. *Appl. Microbiol. Biotechnol.* **1989**, *31*, 272–277.
- (26) Crawford, D. L. Lignocellulose decomposition by selected *Streptomyces* strains. *Appl. Environ. Microbiol.* **1978**, *35*, 1041–1045.
- (27) Hernández-Coronado, M. J.; Hernández, M.; Centenera, F.; Pérez-Leblic, M. I.; Ball, A. S.; Arias, M. E. Chemical characterization and spectroscopic analysis of the solubilization products from wheat straw produced by *Streptomyces* strains grown in solid-state fermentation. *Microbiology* **1997**, *143*, 1359–1367.
- (28) Berrocal, M. M.; Rodríguez, J.; Ball, A. S.; Pérez-Leblic, M. I.; Arias, M. E. Solubilisation and mineralisation of [14 C] lignocellulose from wheat straw by *Streptomyces cyaneus* CECT 3335 during growth in solid-state fermentation. *Appl. Microbiol. Biotechnol.* **1997**, *48*, 379–384.
- (29) Balan, C.; Franco, J. M. Influence of the geometry on the transient and steady flow of lubricating greases. *Tribol. Trans.* **2001**, *44*, 53–58.
- (30) Puls, J.; Schuseil, J. Chemistry of hemicellulose: Relationship between hemicellulose structure and enzyme required for hydrolysis. In *Hemicellulose and hemicellulases*; Coughlan, M. P., Hazlewood, G. P., Eds.; Portland Press: London, U.K., 1993.
- (31) Hernández, M.; Hernández-Coronado, M. J.; Montiel, M. D.; Rodríguez, J.; Pérez, M. I.; Bocchini, P.; Galletti, G. C.; Arias, M. E. Pyrolysis/gas chromatography/mass spectrometry as a useful technique to evaluate the lignolytic action of streptomycetes on wheat straw. *J. Anal. Appl. Pyrolysis* **2001**, *58–59*, 539–551.
- (32) Toledano, A.; Erdocia, X.; Serrano, L.; Labidi, J. Influence of extraction treatment on olive tree (*Olea europaea*) pruning lignin structure. *Environ. Prog. Sustainable Energy* **2013**, *32* (4), 1187–1194.
- (33) Santos, J. I.; Fillat, U.; Martín-Sampedro, R.; Negro, M. J.; Ballesteros, M.; Rodríguez, A.; Eugenio, M. E.; Ibarra, D. Evaluating side-stream lignins from olive tree pruning-based industry: a FTIR and 2D-NMR study. *LignoBIOTECH: 4th Symposium on Biotechnology applied to Lignocelluloses*, Madrid, Spain, Jun. 19–22, 2016; CSIC: Madrid, Spain, 2016.
- (34) Alekhina, M.; Ershova, O.; Ebert, A.; Heikkinen, S.; Sixta, H. Softwood kraft lignin for value-added applications: Fractionation and structural characterization. *Ind. Crops Prod.* **2015**, *66*, 220–228.
- (35) Hernández, M.; Hernández-Coronado, M. J.; Pérez, M. I.; Revilla, E.; Villar, J. C.; Ball, A. S.; Viikari, L.; Arias, M. E. Biomechanical pulping of spruce wood chips with *Streptomyces cyaneus* CECT 3335 and handsheets characterization. *Holzforchung* **2005**, *59*, 173–177.
- (36) Tolbert, A.; Akinosho, H.; Khunsupat, R.; Naskar, A. K.; Ragauskas, A. J. Characterization and analysis of the molecular weight of lignin for biorefining studies. *Biofuels, Bioprod. Biorefin.* **2014**, *8*, 836–856.
- (37) Faix, O. Classification of lignins from different botanical origins by FT-IR spectroscopy. *Holzforchung* **1991**, *45*, 21–28.
- (38) Dorado, J.; Almendros, G.; Field, J. A.; Sierra-Alvarez, R. Infrared spectroscopy analysis of hemp (*Cannabis sativa*) after selected delignification by *Bjerkandera* sp. at different nitrogen levels. *Enzyme Microb. Technol.* **2001**, *28*, 550–559.
- (39) Xiao, B.; Sun, X. F.; Sun, R. Chemical, structural, and thermal characterizations of alkali-soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw. *Polym. Degrad. Stab.* **2001**, *74*, 307–319.
- (40) Ibarra, D.; Chávez, M. I.; Rencoret, J.; Del Río, J. C.; Gutiérrez, A.; Romero, J.; Camarero, S.; Martínez, M. J.; Jiménez-Barbero, J.; Martínez, A. T. Lignin modification during *Eucalyptus globulus* kraft pulping followed by totally chlorine-free bleaching: a two-dimensional nuclear magnetic resonance, Fourier transform infrared, and pyrolysis-gas chromatography/mass spectrometry study. *J. Agric. Food Chem.* **2007**, *55*, 3477–90.
- (41) Griffini, G.; Passoni, V.; Suriano, R.; Levi, M.; Turri, S. Polyurethane coatings based on chemically unmodified fractionated lignin. *ACS Sustainable Chem. Eng.* **2015**, *3*, 1145–1154.
- (42) Lora, J. H.; Glasser, W. G. Recent industrial applications of lignin: A sustainable alternative to nonrenewable materials. *J. Polym. Environ.* **2002**, *10*, 39–48.
- (43) Tejado, A.; Peña, C.; Labidi, J.; Echeverría, J. M.; Mondragon, I. Physico-chemical characterization of lignins from different sources for use in phenol-formaldehyde resin synthesis. *Bioresour. Technol.* **2007**, *98*, 1655–1663.
- (44) Liu, C.; Wang, H.; Karim, A. M.; Sun, J.; Wang, Y. Catalytic fast pyrolysis of lignocellulosic biomass. *Chem. Soc. Rev.* **2014**, *43* (22), 7594–7623.
- (45) Delgado, M. A.; Valencia, C.; Sánchez, M. C.; Franco, J. M.; Gallegos, C. Influence of soap concentration and oil viscosity on the rheology and microstructure of lubricating greases. *Ind. Eng. Chem. Res.* **2006**, *45*, 1902–1910.
- (46) Gupta, R. K. *Polymer and Composite Rheology*, 2nd ed.; Marcel Dekker: New York, NY, USA, 2000.