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Water solubilization of DDGS via derivatization with phosphite esters

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Abstract

Ethanol production from corn starch in the corn dry milling process leaves Distillers' Dry Grains and Solubles (DDGS) as a major by-product from which additional ethanol may be economically obtained from its glucan content. A challenge in processing the cellulose content of this material lies in its extensive inter-cellulose chain hydrogen bonding, which inhibits access of enzymes capable of cleaving glycosidic bonds, a transformation required for providing fermentable sugars. The phosphitylation of cellulosic OH groups using a reactive bicyclic phosphite ester is utilized to disrupt cellulosic hydrogen bonds, thus providing access to cellulose chains for further processing. We describe a method of pretreating DDGS with commercially available trimethylolpropane phosphite $[P(OCH_2)_3CEt]$ in the presence of a slight molar excess of water to afford greater than 90% DDGS solubility in the reaction mixture in methanol and in water. Preliminary results using a model compound [D-(+)-permethylated cellobiose] indicate that glycosidic bonds are cleaved as a consequence of this pretreatment.

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1. Introduction

Methods for the degradation of cellulosic materials to oligosaccharides and sugar alcohols aimed at facilitating ethanol production continue to be the subject of wide and intense interest. Such methods include cellulose treatment with enzymes [mainly cellulases and hemicellulases (Demain et al., 2005; Fan et al., 1987; Zhang and Lynd, 2004)], mineral acids (Mok et al., 1992; Ladisch, 1979, 1989), bases (Ishida et al., 2005), supercritical water (Sasaki et al., 2000), hot water in the presence of a strongly acidic cation exchange resin (Kim et al., 2005; Ladisch et al., 2003), hot water solutions of lanthanide salts (Sakaki et al., 2002), and, more recently, platinum or ruthenium-supported catalysts that accomplish conversion to sugars (Fukuoka and Dhepe, 2006). Other pretreatments studied specifically for DDGS, as well as wet distillers' grains utilize liquid hot water (LHW) or AFEX to make the cellulose more susceptible to hydrolysis as reported in other papers in this special issue (Kim et al., 2008a,b,c).

Approaches to simple disruption of the hydrogen bonds in cellulose have also been described, as for example, hot water treatment (Kobayashi et al., 2005; Kohlmann et al., 1995; Kumagai et al., 2004), pH-controlled hot water treatment (Mosier et al., 2005; Weil et al., 1997; Wyman et al., 2005), extrusion/explosion processing of ammonia-impregnated fibers (AFEX) (Dale et al., 1998, 1999; Teymouri et al., 2005; Liu et al., 2005), steam explosion (Sun et al., 2005; Josefsson et al., 2002; Jain et al., 1999, Wu et al., 1998, 1999), ultrasound treatment (Yang et al., 2004), and dissolution in ionic liquids (Zhu et al., 2006). The use of mixtures of electron-donor solvents with nitrogen oxides, lithium chloride, triethylamine oxide, methylmorpholine oxide, trifluoroacetic acid, orthophosphoric acid, and aqueous solutions of zinc chloride for dissolving cellulose, has been reviewed (Grinshpan, 1998). The intercalating effects of solvents such as Cadoxen or ferric

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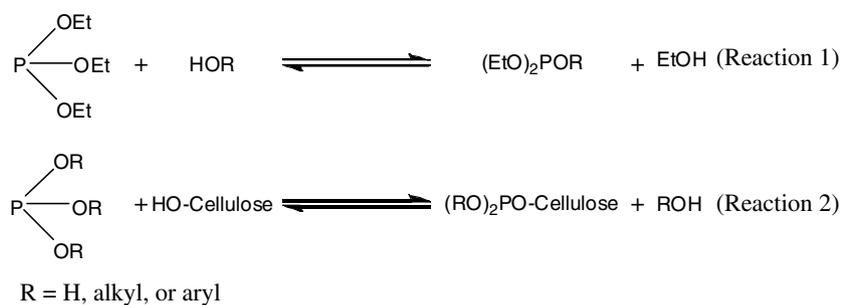
Fig. 1. Reactions of phosphite esters with alcohol or carbohydrate hydroxyl groups.

sodium tartrate have been reported as well (Ladisch et al., 1978, 1992; Hamilton et al., 1984).

In addition to dissolution of cellulosic materials in some of the aforementioned media, some chemical derivatization can and probably does occur, as in the cases of trifluoroacetic and orthophosphoric acids to form trifluoroacetate and phosphate esters, respectively. Dissolving cellulose in an acid anhydride can lead to regioselectively functionalized polymers (El Seoud and Heinze, 2005), and regioselective esterification and etherification of glucose has been demonstrated to influence the processing and use of these products (Burkart et al., 1996). The synthesis of cellulose sulfonates (e.g., tosylates and mesylates) provides polymers with interesting properties as well as intermediates to new cellulosic products (Siegmund, 2002). Fatty acid esters of cellulose lead to novel bioplastics and films (Song et al., 2002; Satge et al., 2004). Such esters also open new synthetic possibilities for introducing functional groups into cellulose to provide pathways to cellulose esters and ethers and their derivatives, as well as biologically active molecules covalently bound to cellulose (Bojanic and Jovanovic, 1998). The reaction kinetics of the production of cellulose ethers (e.g., methyl, hydroxyethylmethyl and hydroxyethyl) have also been reviewed (Doenges, 1990).

As a percentage of the approximately 89% dry matter in Distillers' Dry Grains and Solubles (DDGS) obtained from Big River Resources, LLC, Burlington, IA, cellulose and starch (polyglucoses) comprise ca 16 and 5%, respectively, and the hemicelluloses (polypentoses) xylan, and arabinan comprise a total of about 13.5% (Kim et al., 2008). None of these polysaccharides have appreciable solubility in water, and so it is desirable to develop reasonably mild methods for degrading and/or derivatizing these materials in such a way as to solubilize them in water, since water is the solvent of choice for the commercial production of ethanol by enzyme hydrolysis and fermentation of the resulting sugars (Danner and Braun, 1999). Water solubilization of these polysaccharides and heteropolysaccharides facilitate access to them by cellulases and other hydrolytic enzymes. A recent review (Mosier et al., 2005) describes desired traits in a pretreatment, including its effect on biomass surface area, cellulose crystallinity, and hemicellulose and lignin processability. A coordinated effort to develop leading pretreatment technologies was also reported recently by Wyman et al. (2005).

This paper addresses phosphitylation that has been developed in recent years as a technique for derivatizing carbohydrates, nucleosides and nucleotides (Michalski and Dabkowski, 2004; Dabkowski and Michalski, 2005; Laneman, 2005; Ahmadibeni and Parang, 2005; Oka et al., 2003; Parang et al., 2001), although this technique has been known longer for simple alcohols (Dabkowski et al., 2005; Watanabe et al., 1990). In our efforts to solubilize cellulosic materials with phosphite esters, we have taken advantage of this well-known reaction which is an equilibrium transesterification process in which a new phosphite ester is formed with the release of a new alcohol.

Our efforts described in the present article are focused on developing a novel method for pretreating DDGS which renders the water-soluble product potentially suitable for digestion in animal feed and/or for enzymatic hydrolysis to fermentable sugars for ethanol production after minimal neutralization with base. The economic impact of the value of DDGS (a wet DG) on an ethanol production facility is described by Kim and Dale (2008). Transesterification can be driven by the release of a more volatile alcohol as in reaction 1 in Fig. 1 wherein R is larger than an ethyl group. It has been observed in our laboratories that phosphite esters are also capable of dissolving cellulosic materials to varying degrees (depending on the source) via conversion of at least some of the hydroxyl groups to phosphite ester groups in a phosphitylation reaction (e.g., reaction 2, Fig. 1) the details of which are incompletely understood.

2. Methods

2.1. General

LHW-DDGS was provided by Professor Rick Hendrickson of Purdue University. This material had been prepared by heating wet distillers' grains in light stillage from Big River Resources, LLC to 160 °C for 20 min. Samples of LHW-DDGS were centrifuged and the solids were separated from the liquid solubles. The recovered water-insoluble solids were then dried under vacuum at 45 °C until free flowing dry solids were obtained. Before treatment with phosphites, the dried LHW-DDGS was ground to 0.5 mm. AFEX DDGS was provided by Professor Bruce
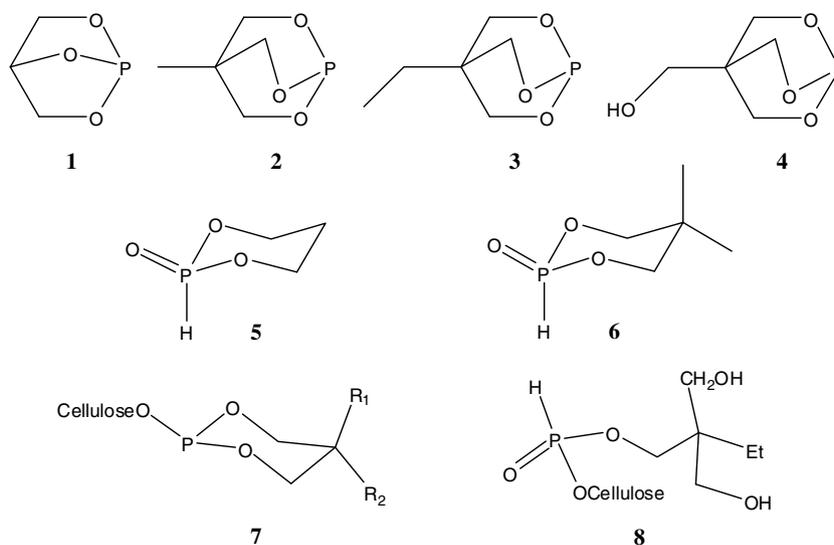
Fig. 2. Phosphite esters **1**–**4**, phosphonates **5** and **6**, and potential products **7** and **8**.

Dale of Purdue University. Lignin was obtained from Westvaco as Indulin AT, a kraft pine lignin polymer. Some of the phosphorus compounds utilized in this study (namely, **1**–**6**) are shown in Fig. 2. Phosphite **1** in this figure was prepared according to a published procedure (Denney and Varga, 1973). Phosphites **2** and **3** in Fig. 2 were also prepared as reported earlier (Wadsworth and Emmons, 1962). Phosphite **3** was a kind gift from Rhodia. Phosphite **4** was prepared according to a literature method (Pike et al., 2004) as were phosphonates **5** and **6** (Fig. 2) (Maffei and Buono, 2003). Phosphorous acid (99%), $(MeO)_3P$ (99+%), $(EtO)_3P$ (98%), $(iPrO)_3P$ (95%), $(PhO)_3P$ (97%), and $(EtO)_2P(O)H$ (94%) were all purchased from Aldrich Chemical Company and were used as delivered.

2.2. Sample procedures for thermal reactions

2.2.1. Treatment of LHW-DDGS with 3

LHW-DDGS (250 mg), **3** (7.14 g) and a small octagonal magnetic stir bar were charged to a 20 mL pressure tube. The tube was sealed with a Teflon cap and submersed in a 100 °C oil bath in order to ensure even heating of the tube and its contents and to avoid sublimation of **3** to cooler parts of the tube. The mixture was stirred for 24 h and then

Fig. 3. Phosphite treatment.

it was allowed to cool to room temperature whereupon 15 mL of methanol was added. Once all the phosphite and derivatized LHW-DDGS were equilibrated with the methanol, the solids were filtered and weighed.

2.2.2. Treatment of LHW-DDGS with 3/water

This reaction was carried out as described in the preceding paragraph except that 1 mL (1.25 equiv.) of water was also charged to the pressure tube.

2.2.3. Treatment of LHW-DDGS with all other phosphites/phosphonates with and without water

These treatments were performed as described in Section 2.2.1 using 250 mg of LHW-DDGS and 44 mmol of the appropriate phosphorus compound. Water (1.25 equiv) was added to the phosphorus compound unless otherwise stated.

2.3. Sample procedure for microwave reactions

LHW-DDGS (1 g), **3** (28.6 g) and a magnetic stir bar, were charged to an 80 mL reaction vessel and the vessel was placed in a CEM Discover microwave reactor. The conditions employed were 300 W, 130 °C, a maximum pressure of 230 psi, and a 6 h reaction time. After the reaction vessel had cooled and was removed from the reactor, its contents were treated in the same manner as described in Section 2.2.1.

3. Results and discussion

3.1. Solubility studies with phosphite esters

A survey of the series of commercially available acyclic phosphite esters $P(OMe)_3$, $P(OEt)_3$, $P(Oi-Pr)_3$ and $P(OPh)_3$ revealed poor solvent properties at temperatures

in excess of 100 °C for LHW-DDGS (and for other cellulosic materials as well) even after prolonged treatment. With the bicyclic phosphite esters **1**–**4** (Fig. 2), however, we encountered more encouraging results. The decision to evaluate these bicyclic esters originated from the observation by one of us in our laboratories (D. G. H.) over 40 years ago that a "boiling stick" (bonded balsa wood) in a beaker containing a reaction mixture for the synthesis of **3** appeared to disintegrate/dissolve.

Although the reaction of **1** with LHW-DDGS produced no appreciable solubilization and results for **4** revealed poor solubility in the same reaction, both **2** and **3** proved interesting. Compounds **1** and **4** are not commercially available but **2** and **3** are. The nature of our initial screening experiments with phosphites **2** and **3** deserves comment at this point. Because these phosphites are viscous liquids at their melting points, thus making filtration difficult at best, we first made qualitative visual estimates of how much LHW-DDGS appeared to be solubilized after pretreatment with these phosphite esters. For that purpose we used a large mass ratio of phosphite to LHW-DDGS in a centrifuge tube having a conical bottom in order to enable visual estimation of how much undissolved material remained in the molten phosphite (see Table 1). When LHW-DDGS solubilization occurs, the molten phosphite changes from clear to yellowish. The solubility in Entry 2 in Table 1 was estimated at approximately 25% reduction in solids by centrifugation of the hot reaction mixture after stirring for 24 h at 80 °C, and the solubility in Entry 4 was estimated at 50% by the same method. From these tests it was determined that **3** was the better medium for solubilizing the LHW-DDGS which exhibited somewhat greater solubility than the AFEX pretreated material.

In order to gain a more quantitative estimate of the solubility of the LHW-DDGS in **3**, the reaction in Entry 4 of Table 1 was repeated except that stirring was continued for 48 instead of 24 h. Because the supernatant from the reaction mixture was soluble in methanol, the reaction mixture was extracted with methanol followed by filtration of the remaining solids. The percent solubility was then calculated from Eq. (1). The solubility of LHW-DDGS in **3** was found to be only 19% (Table 2, entry 1). It should be noted that Eq. (1) does underestimate the amount of LHW-DDGS that solubilized, however, since the elemental phosphorus content increases from 0.47% in the LHW-DDGS to 2.28% in the solid material remaining after reaction of the LHW-DDGS with **3**. In addition, it was observed in

Table 1
Screening of pretreated DDGS solubility in **2** and **3**[a]

Entry	Phosphite	Pretreatment	Solubility observations
1	**2**	AFEX	Partial with small decrease in solute mass; slight tint to the phosphite solution
2	**3**	AFEX	Partial with small decrease in solute mass, but greater than for Entry 1
3	**2**	LHW	Mostly soluble; significant decrease in solute mass; distinct coloration of the phosphite solution
4	**3**	LHW	Mostly soluble; even greater decrease in solute mass than for Entry 3; distinct coloration of the phosphite solution

[a] 3.5% (w/w) of DDGS (35 mg) in **2** or **3** (1 g) was employed. Each sample was heated at 80 °C for 24 h.

Table 2
Optimization of LHW-DDGS Solubilization Protocol[a]

Entry	Phosphite	Lignocellulose solute	Temp./energy source	% Mass change in solute[g]
1	**3**	LHW-DDGS[b]	100 °C	−19
2	**3**	LHW-DDGS[b]	Microwave	−10
3	**4**	LHW-DDGS[b]	100 °C	+15
4	**3**/H$_2$O[c]	LHW-DDGS[b]	100 °C	−92[h]
5	**3**/H$_2$O[c]	LHW-DDGS[b]	80 °C	−86
6	**3**/H$_2$O[c]	LHW-DDGS[b]	150 °C	−99
7	**3**/H$_2$O[c]	LHW-DDGS[b]	Microwave	−74[d]
8	**4**/H$_2$O[c]	LHW-DDGS[b]	100 °C	+750
9	**3**/H$_2$O[c]	AFEX[b]	100 °C	−62
10	**3**/H$_2$O[c]	DG[e]	150 °C	−99
11	**3**/H$_2$O[f]	LHW-DDGS[b]	150 °C	−99

[a] 3.5% (w/w) of lignocellulose solute (250 mg) in phosphite (7.14 g) was employed. Samples were heated at 100 °C for 24 h.
[b] Ground to 0.5 mm particle size.
[c] 7.14 g phosphite/1 mL (1.25 equiv) H$_2$O.
[d] Phosphite solution turned black and the ^{31}P NMR spectrum showed formation of (O)HP(OH)$_2$.
[e] Obtained from Big River Resources, LLC and used with out further treatment; water content ca 41%.
[f] 1:1 mol ratio of phosphite to water pre-reacted at 100 °C prior to addition of lignocellulosic material.
[g] Calculated as the difference in mass of insoluble portions of the solute divided by the original mass of the solute. See text for explanation of the signs.
[h] Avg. of multiple trials ranging from 86% to 97%.

larger scale reactions (ca 1 g of LHW-DDGS and 28.6 g of phosphite) that some precipitation occurred upon methanol extraction. This precipitate, which is presumed to be partially derivatized LHW-DDGS, would add mass to the insolubles and thus further skew the results obtained with Eq. (1). It should be emphasized here that, to the extent that reactions are occurring between **2** and **3** with cellulosic OH groups, these phosphites are behaving as reactive derivatizing solvents as well as conventional solvating solvents.

$$[(\text{Initial LHW} - \text{DDGS Mass}$$
$$- \text{Remaining DDGS Mass})/\text{Initial LHW}$$
$$- \text{DDGS Mass}] \times 100 \qquad (1)$$

3.2. Solubility studies with 3 in the presence of water

Having determined **3** to be the better solubilizing agent, we shifted our focus to developing a standard, optimized treatment. We first looked at using an alternate energy source (Table 2, Entry 2), but microwave irradiation did not afford higher solubility. Presuming that an extra hydroxyl group on the phosphite might afford greater solubility owing to increased disruption of hydrogen bonding among the cellulose chains, **4** was investigated as a solubilizing agent. However, **4** revealed a "positive insolubility" (and hence the positive coefficient for the LHW-DDGS mass change in Entry 3 of Table 2. This result is likely to be due to a derivatization and/or adsorption phenomenon involving surfaces of the solids.

The LHW-DDGS, after drying, contains approximately 8–10% moisture, which may in part account for its better solubility behavior in **3** than the AFEX-DDGS (see above). Thus we were delighted to observe that the addition of 1 mL (0.056 mol, 1.25 equiv) of water to 7.14 g (0.044 mol, 1.00 equiv) of **3** followed by heating to 100 °C resulted in what appeared to be nearly complete dissolution of the 250 mg of LHW-DDGS (Table 2, Entry 4). Addition of methanol to the reaction mixture followed by filtration showed that 92% of the LHW-DDGS had dissolved, leav-

ing a very small amount of an almost colorless insoluble material (LHW-DDGS and/or derivatized LHW-DDGS) which was weighed. Upon evaporation of the methanol from the filtrate, a viscous yellow residue remained. A 200 mg sample of this residue readily dissolved in 2 mL of water at room temperature. The maximum solubility of this product residue in water has yet to be determined.

Decreasing the reaction temperature lowered the solubility (Table 2, Entry 5), while increasing the temperature to 150 °C improved the solubility to nearly quantitative (Table 2, Entry 6). Again, microwave irradiation failed to improve solubility, and the use of **4** with water added to it increased the mass of the insoluble material (Table 2, Entries 7 and 8, respectively). Using **3**/H₂O was also effective for solubilizing AFEX and DDG that had not been treated with hot water (Table 2, Entries 9 and 10, respectively). The result with DDG is significant in that no other pretreatment is necessary when using **3**/H₂O.

On the basis of the foregoing results, it is likely that at least partial hydrolysis of **3** (Bertrand et al., 1974) occurs to produce an active reagent(s) (Scheme 1) for solubilizing cellulose and starch. (The nature of the reaction involving lignin is not clear yet although we have shown that a methylphenyl-ether linkage is probably not cleaved since 1,4-dimethoxybenzene is unreactive with **3** under our conditions.) When **3** is hydrolyzed before adding the LHW-DDGS, the solvent properties are the same as when water is combined with **3** and LHW-DDGS at the same time (Table 2, Entry11). This indicates that hydrolysis of **3** probably occurs faster than its solubilizing action. ³¹P NMR spectroscopic studies demonstrate that in the presence of water, hydrolysis of **3** (δ 94 ppm) produces peaks ranging from 12 to 4 ppm, which is in the phosphonate [O=PH(OR)₂] region of 0–20 ppm (Tebby, 1991). On the other hand, a mixture of methanol and **3** displayed no peaks in the phosphonate region (even after heating to 150 °C) and only the 94 ppm peak corresponding to **3** was still present. Since only 1.25 equiv of water were added to the reaction (Table 2, Entry10 and 11) we suspect that most of the phosphite **3** was hydrolyzed to the phosphonate **9** (Scheme 1) and was not completely hydrolyzed to

Scheme 1. Hydrolysis of **3**.
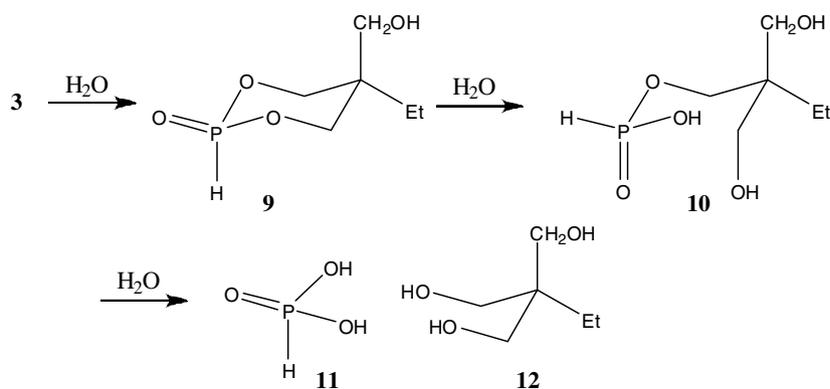
Table 3
LHW-DDGS solubility with varying amounts of water in the presence of 3[a]

Entry	Phosphite (g)	H$_2$O or MeOH (mL)	% Soluble[b]
1	7.14	0.00	19
2	7.14	1.00	99
3	0.50	0.07	86
4	7.14	0.08	37
5	0.93	1.00	89[c]
6	4.50	1.00	100[d]
7	7.14	1.76 MeOH	36

[a] All treatments were performed with 250 mg LHW-DDGS heated at 150 °C for 48 h.
[b] Determined from (weight of insoluble material after methanol extraction)/(initial weight of lignocellulosic material) × 100%.
[c] 10 mol% 3 based on water; reaction mixture turned black.
[d] 50 mol% 3 based on water.

phosphorous acid and trimethylolpropane (11 and 12, respectively, in Scheme 1). This point is of some importance because these results strongly suggest that the phosphite is not merely a source of acid for the well-known acid-catalyzed hydrolysis of polycarbohydrates.

To further investigate the role of water, we conducted a series of experiments in which the amount of water and phosphite were varied (Table 3), and we also employed pure anhydrous phosphorous acid (Table 4, Entry 11) in this study. It is important to note that solubility is poor when no or only a catalytic amount of water is added (Entries 1 and 4, respectively). Entry 3 shows that a substantial quantity of LHW-DDGS material can be solubilized in the 3/H$_2$O mixture since the solution formed is 43% w/w in LHW-DDGS based on the weight of phosphite 3. When excess water is used (Entry 5, Table 3) the result is similar to when pure phosphorous acid is employed (Table 4, Entry 11). Thus the solubility appears to be good but the solution turns black, indicating possibly undesired decomposition.

One of our more promising results is shown in Entry 6 of Table 2 where 3 is used in only 50 mol% relative to water, and yet almost complete solubility is observed. This suggests that less phosphite 3 may be necessary while not sacrificing solubility appreciably. This, however, raises the question of whether 10 (the secondary hydrolysis product of 3) may also be a good or even better solubilizing agent. In two solubility experiments with a 1:1 weight ratio of LHW-DDGS:3, wherein 1 equiv of water was added in one experiment and 2 equiv in the other trial, no significant difference in solubility was observed. These results imply that the dihydrolyzed species 10 in Scheme 1 was equivalent in solubilizing action to that of 9. An approximately equimolar amount of methanol had a measurable effect on the solubility of LHW-DDGS using 3 (Table 3, Entry 7) as opposed to the presence of no added co-solvent (Table 3, Entry 1). The origin of this effect is presently unclear.

Since adding water improved the lignocellulose solubilizing ability of 3, we returned to our study of acyclic phosphites that had earlier revealed poor solubilizing properties

Table 4
Screening of phosphites and phosphonates with LHW-DDGS[a]

Entry	Phosphite	H$_2$O or EtOH	% Soluble[b]
1	MeO—P(OMe)(OMe)	1.25 equiv	86
2	EtO—P(OEt)(OEt)	1.25 equiv	73
3	iPrO—P(OiPr)(OiPr)	1.25 equiv	46
4	PhO—P(OPh)(OPh)	1.25 equiv	31
5	(EtO)(EtO)P(=O)H	0.00 equiv	36
6	(EtO)(EtO)P(=O)H	1.00 equiv EtOH	63
7	cyclic (O)(O)P(=O)H	0.00 equiv	74
8	H$_3$C, H$_3$C cyclic (O)(O)P(=O)H	0.00 equiv	77
9	H$_3$C, H$_3$C cyclic (O)(O)P(=O)H	1.00 equiv EtOH	77
10	cyclic (O)(O)P(=O)H	0.00 equiv	90[c]
11	H(O)P(OH)$_2$	0.00 equiv	86[d]
12	(O=)P(OMe)(OMe)(Me)	0.00 equiv	27
13	(O=)P(OMe)(OMe)(Me)	1.00 equiv	61

[a] All treatments were performed with 250 mg LHW-DDGS heated at 150 °C for 48 h.
[b] Determined from: (weight of insoluble material after methanol treatment)/(initial weight of lignocellulosic material) × 100%.
[c] 80 °C.
[d] Solids and solution blackened.

by themselves, in order to investigate the effectiveness of adding water to them. We also wished to examine several phosphonates that resemble compound 5. Such compounds

could prove to be solubilizing solvents that could rival **3**/H_2O. The results summarized in Entries 1–4 of Table 4 demonstrate that solubilities were indeed improved by the presence of water, but the solubilities realized were inferior to those achieved with **3**/H_2O. The good to excellent solubilities observed with the cyclic phosphonates (Table 4, Entries 7–10) supports our hypothesis that one or more hydrolyzed products of **3** are important in providing high solubilities of lignocellulosic material.

It was observed that the acyclic candidate diethylphosphonate (Table 4, Entry 5) did not provide LHW-DDGS solubility as great as a mixture of triethyl phosphite and 1.25 equiv of water (Entry 2) although these solubilizing systems might have been expected to be approximately equivalent solvent systems according to our original hypothesis. However, the hydrolysis of triethyl phosphite also produces ethyl alcohol which was shown to enhance solubilization in the presence of diethylphosphonate (see Entries 5 and 6). On the other hand, however, addition of 1 equiv of ethanol to 2,2-dimethyl-1,3-propane phosphonate (Entry 9) showed no effect on solubility. It is apparent that these observations comprise a complex phenomenon requiring additional study to understand the role of ethanol in the solubilization using triethyl phosphite. The possibility that the superior solubilization properties of **3**/H_2O may be attributed to the hydroxyl group present on a molecule of **9** (monohydrolyzed **3**) should also be investigated. To confirm that hydrolyzed phosphites **9** and **10** are not merely sources for phosphorous acid, we employed commercially available phosphorous acid (**11**, 99% pure crystalline solid) as a solubilizing agent for LHW-DDGS and also for lignin (Entry 11, Table 4, and Entry 9, Table 5, respectively). As seen in these entries, phosphorous acid was quite effective in solubilizing LHW-DDGS and it even partially dissolved lignin. However, in both cases, the reactions produced black solutions and black residues. Although this result is not surprising in the case of the lignin experiment (since the lignin used was black-brown) blackening of the tan LHW-DDGS sample is likely due to undesirable decomposition. The alkyl phosphonate methyldimethylphosphonate in Entry 12 of Table 4 solubilized only 27% of the LHW-DDGS sample, while the same experiment carried out with the addition of 1.00 equiv of water produced 61% solubility (Entry 13) implying that the relatively small solubility observed in Entry 12 might be due to hydrolysis of one of the –OMe ester groups of the alkyl phosphonate by moisture in the LHW-DDGS and/or in the alkyl phosphonate itself.

It is interesting to note that our observation of 99% solubility of LHW-DDGS in **3**/H_2O is over 60% more than the total 34.5% carbohydrate analysis (Kim et al., 2008) indicating substantial solubilization of other constituents by **3**/H_2O. Since spectroscopic analysis of the complex mixture we produce is extremely difficult, we studied some model systems in an effort to elucidate some of the chemistry that was occurring in the solubilization process. To

Table 5
DDGS model component solubility[a]

Entry	Component	Phosphite	Water	Solubility[b]
1	Cellulose[c]	**3**	1.25 equiv	99
2	Cotton	**3**	1.25 equiv	97
3	Cellobiose[d]	**3**	1.25 equiv	99[j]
4	Me-cellobiose[e]	**3**	1.25 equiv	99[j]
5	Xylan[f]	**3**	1.25 equiv	99
6	Lignin[g]	**3**	1.25 equiv	99
7	Zein[h]	**3**	1.25 equiv	99
8	Corn oil[i]	**3**[k]	1.25 equiv	31
9	Lignin[g]	**11**	0.00 equiv	15[l]

[a] All treatments were performed with 250 mg of component and 44 mmol of phosphite heated at 150 °C for 48 h.
[b] Determined from: (weight of insoluble material after methanol treatment)/(initial weight of lignocellulosic material) × 100%.
[c] Chromatography grade from Aldrich.
[d] D-(+)-cellobiose from Aldrich.
[e] Permethylated according to Mendonca and Laine, Carbohydr Res. 2005, 2055-59.
[f] From oat spelts (Aldrich).
[g] Indulin AT kraft pine lignin from Westvaco.
[h] From Aldrich.
[i] Grocery store salad grade corn oil.
[j] 100 °C.
[k] One g of corn oil:1 g of **3**.
[l] Reaction mixture turned black.

insure that we were indeed solubilizing the cellulose content of LHW-DDGS, we examined cellulose, cotton and cellobiose as substrates. As expected, all gave very good solubilities (Table 5, Entries 1–3). Permethylation of the cellobiose did not effect the solubility (Entry 4), suggesting perhaps that glycosidic bond breaking was occurring, further evidence for which will be discussed below. We would not expect selectivity for certain carbohydrates in our process, and this is illustrated by the almost complete dissolution of xylan, another carbohydrate component of LHW-DDGS (Entry 5). As a percentage of the ca 89% dry mass content of DDGS, low molecular weight phenolics make up about 6–7%. Although these materials are not useful for conversion to ethanol, we showed that pine lignin is also highly soluble in **3**/H_2O (Table 5, Entry 6) and interestingly, the material remaining after methanol extraction and evaporation also was water-soluble. It should be emphasized again that, because of the possibility of reactions involving transesterification with cellulosic OH groups, these phosphites are poised to behave as reactive solvents as well as conventional solvating solvents (see also later). The excellent solubility of Zein seen in Entry 7 of Table 5 implies that the protein content of the DDGS is undoubtedly also solubilized. This observation may be important in considering separations downstream. However, any protein phosphitylation that may occur would have to be analyzed for compatibility with livestock if such materials were to be used as a high-protein feed additive.

Corn oil (Entry 8 in Table 5) was only 31% soluble in an equal mass of **3**/H_2O, and interestingly, the un-dissolved corn oil appeared to be decolorized. Since filtration was

not a viable option, methanol was added to dissolve polar materials in the reaction mixture, leaving the remaining oil as a separate phase which was then extracted into hexanes. Separation of the hexanes from the methanol solution phase followed by evaporation under reduced pressure of the hexanes extract left a colorless oil containing no phosphorus detectable by P^{31} NMR spectroscopy. A ^1H NMR spectrum of this oil indicated that the glycerol backbone of the oil had undergone a reaction. It is conceivable that the mildly acidic hydrolysis product **9** (Scheme 1) facilitates hydrolysis of the triglyceride with the excess 0.25 equiv of water to form diglyceride plus free fatty acid. Such hydrolysis could be acid-catalyzed via the phosphite tautomer of **9** depicted on the left side of reaction 3 in Fig. 4. Indeed, an FT-IR spectrum of the colorless oil revealed an –OH peak that could be attributed to the presence of diglyceride. The free fatty acids liberated during hydrolysis of triglyceride to diglyceride should be soluble in methanol, and this process could account for the 31% solubility of the oil in the **3**/H_2O medium, assuming that the refined corn oil we used is composed of ca 99% triglycerides (Moreau and Hicks, 2004). Methanol was removed from the MeOH-soluble portion of the reaction mixture leaving a viscous yellowish oil that was analyzed by P^{31} NMR and H^1 NMR spectroscopies. The P^{31} NMR spectrum showed phosphorus signals attributable to unreacted **9** and **10**, and also multiple minor peaks due to unidentified phosphorus species in the 0–20 ppm range. This range could be associated with various phosphitylated components of the corn oil. The ^1H NMR spectrum corroborated the P^{31} NMR spectroscopic results by displaying minor unidentified peaks and high intensity peaks due to the presence of **9** and **10**. The fact that there

were no phosphorus peaks in the colorless oil extracted into hexanes implies that a value-added usable corn oil co-product might be obtained using our treatment protocol.

It is likely that when a large excess of phosphite **3**/H_2O is employed to achieve 99% LHW-DDGS solubility that the oil content will be completely hydrolyzed and therefore soluble in methanol. However, in cases such as Entry 3 of Table 3, where lower phosphite:LHW-DDGS ratios are used, the lower solubility encountered could be due, at least in part, to insoluble oil present in the LHW-DDGS. This may be promising since lower mass of phosphite usage is desired from an economical standpoint. Thus if lower biomass solubility is primarily due to an increase in insoluble materials that do not contain an economically significant source of fermentable sugars, the excess phosphite required to obtain 99% biomass solubility would not be necessary to obtain economically viable yields of fermentable sugars as well as potentially value-added feed product.

An additional potential contributing factor to the solubility of the LHW-DDGS is contained in Scheme 1 and reaction 3 of Fig. 4 in which are illustrated the formation of a donor-solvent/additive mixture somewhat analogous to such mixtures referred to in the introduction in which electron-donor solvents (e.g., DMSO) with the additives trifluoroacetic or orthophosphoric acid have been used to dissolve cellulose (Grinshpan, 1998). In our solubilizing solvent system **3**/H_2O, each reactant and product in Scheme 1 is an electron pair donor solvent component since each possesses at least three oxygens, each of which bears two lone pairs of electrons. Moreover, **3** features a phosphorus lone pair and the same is true for the tautom-
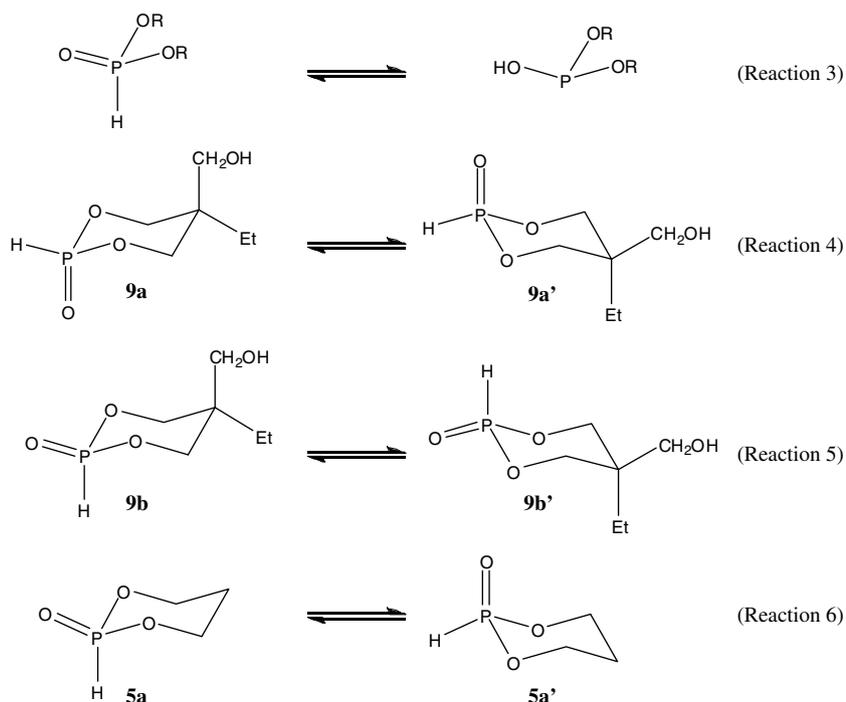
Fig. 4. Tautomer, isomer and conformation changes in hydrogen phosphonates.

ers of **9**–**11** because of the well-known tautomeric equilibrium generalized for **9**–**11** in reaction 3 of Fig. 4. In this equilibrium, the phosphorus atom in the tautomer on the right has an electron lone pair while the one on the left does not. It should be noted, however, that this equilibrium does lie far to the left. It is seen in Scheme 1 that each species featuring an O=P–H moiety can exist as an HOP tautomer (see reaction 3 in Fig. 4) which being acidic can liberate a proton. The HOP tautomers therefore constitute "additives" analogous to the aforementioned acids.

3.3. Spectroscopic studies

3.3.1. Reactions of DDGS with 3

A ^{31}P NMR spectrum (in CD_3OD) of the solid residue remaining after evaporation of the methanol extract in the treatment of LHW-DDGS with **3** showed a single peak at 94 ppm corresponding to **3** (in CD_3OD or CD_3Cl). In addition, there were minor peaks between 0 and 20 ppm, the region in which monoalkyl and dialkyl phosphites typically appear (Tebby, 1991). This is consistent with the presence of phosphitylated LHW-DDGS as will be further discussed in the next section. If the methanol had caused significant transesterification of **3** (or of phosphitylated LHW-DDGS) $P(OCH_3)_3$ would have been formed, with the appearance of a corresponding ^{31}P NMR peak at 140 ppm. However, no peaks were observed in this region.

3.3.2. Reactions of DDGS with 3 in the presence of water

^{31}P NMR spectra of product mixtures resulting from treatment of LHW-DDGS with **3** compared with those from the reaction of **3** with 1.00 equiv. of water added, and those resulting from treatment with a mixture of **3** and 1.25 equiv of water, were all complex and quite similar in appearance. However, the latter two reaction mixtures displayed (as expected) no peak at 94 ppm corresponding to **3**. Because of similarities among the phosphorus O-alkyl substituents in the hydrolysis products of **3**, and in the products of transesterification reactions of **9** with cellulosic OH groups, it was difficult to distinguish which ^{31}P signals corresponded to phosphite species **9a**, **a′**, **b**, **b′** resulting from the conformer/isomer equilibria (Mosbo and Verkade, 1973) shown in reactions 4 and 5 in Fig. 4, which ^{31}P resonance was due to **10**, and which peaks represented phosphite groups on derivatized LHW-DDGS depicted as **7** in Fig. 2. We thus synthesized **5** and **6** (Fig. 2) as model compounds for the first hydrolysis intermediate **9** in Scheme 1. A ^{31}P spectrum of pure **5** in deuterated methanol reveals part of the reason for the complexity of ^{31}P NMR spectra of **3**/H_2O/cellulosic material mixtures. First, an approximately 5.4 ppm difference in chemical shift is observed between the two phosphorus resonances (12.4 and 7.0 ppm in CH_3OH) assignable to the presence of the two conformers of **5** shown in reaction 6 in Fig. 4. These conformers exist in an equilibrium which is slow on the NMR time scale in CH_3OH but is either fast in $CDCl_3$, or else this solvent greatly favors one of the conformers

wherein its resonance appears as a single peak at 4.1 ppm. Secondly, deuterium/hydrogen exchange equilibria between the OD deuterium in CD_3OD and the P–H bonds in **5**, **9a**, **a′**, **b**, and **b′** cause additional ^{31}P NMR spectral features to appear owing to P-D spin-spin coupling. Thus the nuclear spin of ^{2}D is 1 and each ^{31}P NMR resonance of **5** appears as a triplet of three equally spaced lines of equal intensity $[^1J(^{31}P^{-2}D) \approx 106\ Hz]$. ^{31}P NMR spectra of pure **5** in MeOH with a $CDCl_3$ lock inserted showed a simple spectrum of only two peaks (12.4 ppm and 7.0 ppm) corresponding to the two conformers shown in reaction 6, since P-D spin-spin coupling was necessarily absent. Thirdly, as noted above, the ^{31}P NMR spectrum of **9** (the initial hydrolysis product of **3**) gains additional complexity owing to the presence of two conformations in a slow equilibrium with each other for *each isomer* of **9** (namely, **9a**, **a′** and **9b**, **b′**) as shown in reactions 4 and 5 of Fig. 4.

The aforementioned issues also complicated the CD_3OD-solution ^{31}P NMR spectra of the viscous product left after evaporation of the methanol extract of 99% LHW-DDGS solubilized in excess **3**/1.25 equivwater. In order to enhance the intensity of the phosphitylated LHW-DDGS resonances in the ^{31}P NMR spectra, 1.0 g of this material was reacted with 1.0 g of **3** and 0.14 g (1.25 eq) of water with the aim of reacting all of the phosphite using an excess of LHW-DDGS. About half of the LHW-DDGS was solubilized (0.59 g) not only suggesting that all the phosphite that could be utilized to solubilize the LHW-DDGS had indeed been utilized under our conditions, but also indicating that at least a 50 w/w% solution of LHW-DDGS in an approximately equimolar mixture of phosphite **3** and water could be achieved. The latter pleasing result portends well for potential economical industrial processing using our pretreatment protocol (provided that phosphite can be economically recovered for recycling – see below). A ^{31}P NMR spectrum in CH_3OH of the resulting product revealed phosphorus signals from 0 to 20 ppm, but no peak at 94 ppm for **3**. There were multiple peaks (0–20 ppm) as would be expected for such a mixture. Some of these peaks were new, some corresponded to unreacted **9**, but none could be unambiguously identified as a single derivative. It is clear that considerable additional work is required with components separated from this complex solution of DDGS in order to determine which ones are being derivatized and which are merely being solubilized. However, our efforts on model substrates mentioned above may help us solve some of these spectral problems in the near future.

In an attempt to shed further light on the solubilization process of LHW-DDGS by our **3**/H_2O protocol, we reacted D-(+)-permethylated cellobiose with **5** (which functioned as a solvent comparable to **3**/water for LHW-DDGS) in a 1:1 molar ratio. The virtual absence of hydroxyl groups in **5** (owing to the very minor concentration of the second tautomer) and the unreactivity of **3**/H_2O with ether linkages restricts any reaction under our conditions

to cleavage of the glycosidic bond of D-(+)-permethylated cellobiose. In addition to ^{31}P NMR peaks characteristic of unreacted **5** (12.4 and 7.0 ppm) two additional ^{31}P NMR peaks appeared; a major resonance at 11.2 ppm and a minor one at 7.9 ppm. The presence of the major resonance is consistent with glycosidic bond cleavage, since the D-(+)-permethylated cellobiose was determined via FTIR spectral analysis to contain no significant amount of free –OH groups or water. The minor peak ^{31}P NMR at 7.9 ppm is thought perhaps to be due to reaction of **5** with free –OH impurities owing to incomplete methylation of cellobiose in concentrations sufficiently low to be undetectable by FTIR spectroscopy.

The ^{31}P NMR spectra of the reaction product of LHW-DDGS with **3**/H_2O mixtures (which presumably produced **9**) also exhibited peaks in the 11 ppm region consistent with glycosidic bond cleavage. Because the glycosidic linkage is quite robust, we inferred that there might be evidence in the ^{31}P NMR spectra of the esterification product stemming from reaction of POH/[P(O)H] tautomeric groups in **5** and in **9** with one or more different cellulose OH groups in the DDGS to give **8**. As stated earlier, however, ^{31}P NMR chemical shifts between 75 and 162 ppm typical for such phosphite esters (Tebby, 1991) were not observed. We then conjectured that glycosidic bond cleavage arose via acid-catalysis by phosphorous acid formed via hydrolysis in DDGS/**3**/H_2O reactions. Such hydrolysis can also be ruled out, however, at least in the reaction of **5** with D-(+)-permethylated cellobiose mentioned above, since there are peaks in the ^{31}P NMR spectra of the reaction products (4–

13 ppm) which are consistent with the formation of new dialkyl phosphonate species (−2.5 to +22.5 ppm, Tebby, 1991) which can be associated with glycoside bond cleavage by **5**. Moreover, no water is present in the reaction, which would be required for hydrolysis of the D-(+)-permethylated cellobiose.

On the basis of these arguments, we believe that glycosidic linkages are cleaved, and a plausible mechanism is shown in Fig. 5. Nucleophilic attack on the phosphorus by the glycosidic oxygen lone pair [analogous to acetate oxygen attack of a dialkylphosphonate (Luz and Silver, 1962)] facilitates cleavage of the glycosidic bond to produce a six-membered ring carbocation of one glucose unit that is known to occur during acid-catalyzed hydrolysis (Xiang et al., 2003). Subsequent equilibrium reactions eventually allow the ring of the cyclic dialkyl phosphonate to open and the resulting oxyanion could then attack the cyclic carbocation to give a dialkylphosphite bonded to two glucose acetals as shown in Fig. 5. It is also conceivable that a four-membered ring intermediate is formed as shown in **B** of Fig. 5, despite increased steric congestion that could be involved.

3.4. A rationale

Our original goal was to develop a phosphitylating solvent/reagent for efficiently disrupting the robust hydrogen bonding occurring between strands of native cellulose polymers, thus causing solubilization. Phosphitylating agents **3** and **4** were envisioned to accomplish this objective by
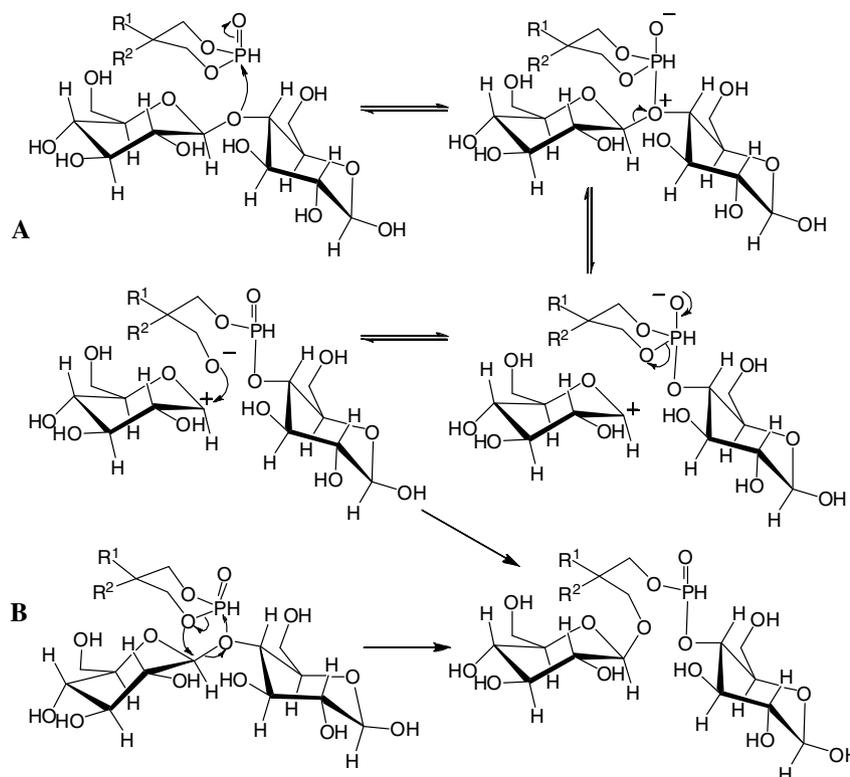
Fig. 5. Plausible mechanism for phosphonate cleavage of glycosidic bonds in cellulose by either a stepwise (**A**) or a concerted (**B**) reaction.
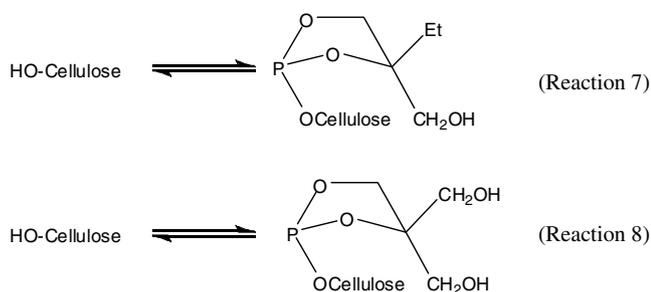
Fig. 6. Originally hypothesized reactions of **3** and **4** with cellulose which are apparently not major derivatization pathways according to P^{31} NMR spectroscopic evidence.

reacting with cellulose to form one new OH group as in reaction 7 (see Fig. 6) by employing **3**, or two new OH groups as in reaction 8 (see Fig. 6) with the use of **4**. Our postulate was that one or two OH groups in the different chemical environments provided by their new more random locations might better hydrogen bond with water than with cellulose hydroxyl groups. With our discovery that **3** is a better solvent/reagent than **4**, along with the observation that the presence of water greatly improves the solubility of DDGS in **3**, the possibility that phosphorous acid (**11**) would function as an efficient derivatizing agent existed. This seems to be suggested by the results in Entry 11 of Table 4 and Entry 9 of Table 5.

Although phosphorous acid may play a partial role, the observation that **3** appears to be a better solvent for DDGS than **2** or **4** indicates that the bicyclic phosphite does not simply serve as a source of phosphorous acid in the presence of water. Supportive of this view is the apparent decomposition observed with phosphorous acid (Entry 5 of Table 3, Entry 11 of Table 4, and Entry 9 of Table 5) compared with the results obtained with a mixture of **3**/H$_2$O. It is also interesting that an analogous mixture of **4**/H$_2$O did not provide good solubility despite the presence of the extra OH group which we originally postulated would enhance the ability of **4** to hydrogen bond with water or methanol. In fact, the use of **4** led to a significant increase in the mass of the insoluble portion of the LHW-DDGS, presumably because of derivatization.

We believe that **9** (which is formed first via hydrolysis of **3** in Scheme 1) acts as an active phosphitylating/solubilizing agent. If this is true, it may not be necessary to completely remove the water from the light stillage. Indeed we have observed that un-pretreated Distillers' Grains (DG) which has a significant moisture content, showed good solubility (Table 2, Entry 10).

4. Solvent/reactant recovery

4.1. Reactions of DDGS with **3**

Compound **3** is an easily sublimable solid. After evaporation of the methanol extract of the reaction mixture produced by heating DDGS in **3**, approximately 67% of **3** was recovered (Fig. 3). Recovery of additional **3** should be possible since the ^{31}P NMR spectrum of the residue remaining after sublimation displayed a prominent peak for **3**. We have also experienced some success in recovering unreacted **3** by ether extraction (Fig. 3). However, the ether layer also contained other phosphorus-containing species as indicated by ^{31}P NMR spectroscopy, and their identities are presently unclear. In addition, the ether-insoluble portion also revealed the presence of a significant amount of **3** via ^{31}P NMR spectroscopy.

4.2. Reactions of DDGS with **3** in the presence of water

As noted earlier in this paper, a reaction mixture of **3** and water upon heating produces EtC(CH$_2$OH)$_3$ and (O)HP(OH)$_2$ (**11**) in an equilibrium reaction. Phosphite **3** can be partially regenerated by elimination of water upon heating in the presence of a catalytic amount of triethylamine. Initial efforts at recovering **3** by distillation/sublimation from the aforementioned product mixture were difficult because **3** seems to co-distill with water, giving a distillate consisting of a mixture of **3** and hydrolysis products **9** and **10**. However, this does not pose a major concern since the distillate could be recycled.

Further work aimed at answering the many questions remaining, such as phosphite recoverability, are underway. It is also conceivable that the poorer solubilization properties of acyclic phosphites relative to **3** could be offset by their lower cost. It is clear that downstream testing with different enzymatic saccharification protocols with subsequent or simultaneous fermentation will be necessary to determine the optimum solubilizing system. Factors to be considered in such tests will include solubility performance, sugar yield, phosphite recoverability demands/limits, costs associated with materials and energy required. It may even be the case that phosphorous esters other than **3** affording poorer solubility using lower temperatures, for example, would be more compatible with downstream processing into ethanol and/or other products.

Acknowledgement

References

Ahmadibeni, Y., Parang, K., 2005. Solid-phase reagents for selective mono-phosphorylation of carbohydrates and nucleosides. J. Org. Chem. 70, 1100–1103.

Bertrand, R.D., Berwin, H.J., McEwen, G.K., Verkade, J.G., 1974. Stereochemistry of Cage Opening in P(OCH$_2$)$_3$CMe Hydrolysis. Phosphorus 4, 81–89.

Bojanic, V., Jovanovic, S., 1998. Synthesis of new materials by chemical modification of cellulose. Tabakovic, I. Hemisjska Industrija 52, 191.

Burkart, P., Wagenknecht, W., Nehls, I., Klemm, D., Stein, A., Heinze, T., 1996. Regioselective derivatization of cellulose. Routes of synthesis, effects on properties, and areas of application. Polym. News 21, 155.

Dabkowski, W., Michalski, J., 2005. A novel approach to synthesis of nucleotides and their structural analogues via tricoordinate phosphorus reagents. Collection Symposium Series 7 (Chemistry of Nucleic Acid Components), pp. 39–46.

Dabkowski, W., Ozarek, A., Tworowska, I., 2005. Highly selective O-phosphitylation of amino alcohols using PIII-reagents containing 4-nitro and 2,4-dinitro aryloxy leaving groups. New J. Chem. 29, 11.

Dale, B.E., Weaver, J., Byers, F.M., 1999. Extrusion processing for ammonia fiber explosion (AFEX), Applied Biochem. Biotechnol. vols. 77–79.

Dale, B.E., Weaver, J., Byers, F.M., 1998. In: Twentieth Symposium on Biotechnology for Fuels and Chemicals. Humana Press Inc., pp. 35–45.

Danner, H., Braun, R., 1999. Biotechnology for the production of commodity chemicals from biomass. Chem. Soc. Rev. 28, 395.

Demain, A.L., Newcomb, M., Wu, J.H.D., 2005. Cellulase, Clostridia, and Ethanol. Microbiol. Molecul. Biol. Rev. 69, 124.

Denney, D.B., Varga, S.L., 1973. Preparation and chemistry of 2,6,7-trioxa-1-phosphabicyclo[2.2.1]heptane. Phosphorus and the Related Group V Elements 2, 245–248.

Doenges, R., 1990. Non-ionic cellulose ethers. British Polym. J. 23, 315–326.

El Seoud, O.A., Heinze, T., 2005. Organic esters of cellulose: new perspectives for old polymers. Adv. Polym. Sci. 186 (Polysaccharides I), 103.

Fan, L.T., Gharpuray, M.M., Lee, Y.-H., 1987. Cellulose Hydrolysis. Springer, Berlin.

Fukuoka, A., Dhepe, P.L., 2006. Catalytic conversion of cellulose into sugar alcohols. Angew. Chem. Int. Ed. 45, 5161.

Grinshpan, D.D.B., 1998. Novel processes for production and processing of cellulose solutions. In: Sviridov, B.B. (Ed.), Khimicheskie Problemy Sozdaniya Novykh Materialov I Tekhnologii, vol. 87. Belorusskii Gosudarstvennyi Universitet, Minsk.

Hamilton, T.J., Dale, B.E., Ladisch, M.R., Tsao, G.T., 1984. Effect of ferric tartrate/sodium hydroxide solvent pretreatment on enzyme hydrolysis of cellulose in corn residue. Biotechnol. Bioeng. 26, 781–787.

Ishida, M., Otsuka, K., Takenanka, S., Yamanaka, I., 2005. One-step production of CO- and CO_2-free hydrogen from biomass. J. Chem. Technol. Biotechnol. 80, 281.

Jain, R.K., Ibrahim, M., Wright, R.S., Glasser, W.G., 1999. Biopolymers by steam explosion/fractionation: cellulose, lignin, xylan and derivatives. Book of Abstracts, 218th ACS National Meeting, New Orleans, Aug. 22–26 CELL-041.

Josefsson, T., Lennholm, H., Gellerstedt, G., 2002. Steam explosion of aspen wood. Characterization of reaction products. Holzforsch. 56 (3), 289–297.

Kim, Y., Hendrickson, R., Mosier, N., Ladisch, M.R., 2005. Plug flow reactor for continuous hydrolysis of glucans and xylans from pretreated corn fiber. Energy Fuels 19, 2189–2200.

Kim, S., Dale, B.E., 2008. Life cycle assessment of fuel ethanol derived from corn grain via dry milling. Bioresour. Technol. 99, 5250–5260.

Kim, Y., Mosier, N.S., Hendrickson, R., Ezeji, T., Blaschek, H., Dien, B., Cotta, M., Dale, B., Ladisch, M.R., 2008a. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. Bioresource Technol. 99, 5165–5176.

Kim, Y., Hendrickson, R., Mosier, N., Ladisch, M.R., Bals, B., Balan, V., Dale, B.E., 2008b. Enzyme hydrolysis and ethanol fermentation of liquid hot water and AFEX pretreated distiller's grains at high solids loadings. Bioresour. Technol. 99, 5206–5215.

Kim, Y., Mosier, N.S., Ladisch, M.R., 2008c. Process simulation of modified dry grind ethanol plant with recycle of pretreated and enzymatically hydrolyzed distiller's grains. Bioresour. Technol. 99, 5177–5192.

Kobayashi, N., Sato, T., Okada, N., Hatano, S., Itaya, Y., Kobayashi, J., Mori, S., 2005. The liquefaction technique of pulverized super fine ligneous biomass powder by hot compressed water treatment. In: World Congress of Chemical Engineering, 7th, Glasgow, United Kingdom, July 10–14.

Kohlmann, K., Westgate, P.J., Sarikaya, A., Velayudhan, A., Weil, J., Hendrickson, R., Ladisch, M.R., 1995. Enhanced enzyme activities on hydrated lignocellulosic substrates, BTEC Paper 127, 207th American Chemical Society National Meeting, ACS Symposium Series No. 618, Enzymatic Degradation of Insoluble Carbohydrates, pp. 237–255.

Kumagai, S., Hayashi, N., Sakai, T., Nakada, M., Shibata, M., 2004. Fractionation and saccharification of cellulose and hemicellulose in rice hull by hot-compressed-water treatment with two-step heating. J. Jpn. Inst. Energy 83 (10), 776–781.

Ladisch, M.R., Ladisch, C.M., Tsao, G.T., 1978. Cellulose to sugars: new path gives quantitative yield. Science 2001, 743–745.

Ladisch, M.R., 1989. Hydrolysis. In: Hall, C.W., Kitani, O. (Eds.), Chapter in Biomass Handbook. Gordon and Breach, London, pp. 434–451.

Ladisch, M.R., 1979. Fermentable sugars from cellulosic residues. Process Biochem. 14 (1), 21–25.

Ladisch, M.R., Waugh, L., Westgate, P., Kohlmann, K., Hendrickson, R., Yang, Y., Ladisch, C., 1992. Intercalation in the pretreatment of cellulose in harnessing biotechnology for the 21st century. In: Ladisch, M.R., Bose, A. (Eds.), Proceedings of the Ninth International Biotechnology Symposium and Exposition. American Chemical Society, Washington, DC, pp. 510–518.

Ladisch, M.R., Mosier, N., Hendrickson, R., Dreschel, R., Welch, G., Dien, B.S., Bothast, R. 2003. Principles and Economics of Pretreating Cellulose in Water for Ethanol Production. Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, March 23–27, AGFD-103.

Laneman, Scott A., 2005. Phosphorylation & phosphitylation: a mini review. Specialty Chem. Mag. 25 (1), 30–32.

Liu, N., Shi, S., Xiandai, H., 2005. Research progress of converting lignocellulose to produce fuel ethanol. Modern Chemical Industry 25 (3), 19–22, 24.

Luz, Z., Silver, B., 1962. N.M.R. study of the exchange of phosphorus bonded hydrogen in dimethyl- and diethylphosphonate. Catalyzed by Acetate Ion 84, 1095–1098.

Maffei, M., Buono, G., 2003. A two step synthesis of 2-oxo-2-vinyl 1,2,3-dioxaphospholanes and -dioxaphosphorinanes. Tetrahedron 59, 8821–8825.

Michalski, J., Dabkowski, W., 2004. State of the art chemical synthesis of biophosphates and their analogues via PIII derivatives. Topics Curr. Chem. (New Aspects in Phosphorus Chemistry IV) 232, 93–144.

Mok, W.S., Antal, M.J., Varhergyi, G., 1992. Productive and parasitic pathways in dilute acid-catalyzed hydrolysis of cellulose. Ind. Eng. Chem. Res. 31, 94.

Moreau, R.A., Hicks, K.B., 2004. The composition of corn oil obtained by the ethanol extraction of ground corn. In: Proceedings, Corn Utilization and Technology Conference, June 7–9, 2004. Indianapolis, IN, Poster 1.

Mosbo, J.A., Verkade, J.G., 1973. Chemical implications of phosphorus configuration in isomeric 2-substituted 2-oxo-1,3,2-dioxaphosphorinanes. J. Am. Chem. Soc. 95, 204–209.

Mosier, N.S., Hendrickson, R., Brewer, M., Ho, N., Sedlak, M., Dreschel, R., Welch, G., Dien, B.S., Aden, A., Ladisch, M.R., 2005a. Industrial scale-up of ph controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. Appl. Biochem. Biotech. 125, 77–97.

Mosier, N., Wyman, C.E., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005b. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96 (6), 673–686.

Mosier, N.S., Hendrickson, R., Brewer, M., Ho, N.W.Y., Sedlak, M., Dreschel, R., Welch, G., Dien, B.S., Aden, A., Ladisch, M.R., 2005c. Industrial scale-up of pH-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. Appl. Biochem. Biotechnol. 125, 77–85.

Oka, N., Wada, T., Saigo, K., 2003. An oxazapholidine approach for the stereocontrolled synthesis of oligonucleoside phosphorothioates. J. Am. Chem. Soc. 125 (27), 8307–8317.

Parang, K., Fournier, Eric J.-L., Hindsgaul, O., 2001. A solid phase reagent for the capture phosphorylation of carbohydrates and nucleosides. Org. Lett. 3 (2), 307–309.

Pike, R.D., Reinecke, B.A., Dellinger, M.E., Wiles, A.B., Harper, J.D., Cole, J.R., Dendramis, K.A., Borne, B.D., Harris, J.L., Pennington, W.T., 2004. Bicyclic phosphite esters from pentaerythritol and dipentaerythritol: new bridged ligands in organometallic and inorganic chemistry. Organometallics 23, 1986–1990.

Sakaki, T., Shibata, M., Seri, K., Inoue, Y., Ishida, H., 2002. Jpn. Kokai Tokkyo Koho CAN 136:246813.

Sasaki, M., Fang, Z., Fukushima, Y., Adschiri, T., Arai, K., 2000. Dissolution and hydrolysis of cellulose in subcritical and supercritical water. Ind. Eng. Chem. Res. 39, 2883.

Satge, C., Granet, R., Verneuil, B., Branland, P., Krausz, P., 2004. Synthesis and properties of biodegradable plastic films obtained by microwave-assisted cellulose acylation in homogeneous phase. Compt. Rend. Chim. 7, 135.

Siegmund, G., 2002. Cellulose–cellulose sulfonates: preparation, properties, subsequent reactions Klemm, D. Polym. News 27, 84.

Song, L., Ma, F., Shao, Z., Tan, H., 2002. Use of a Co-reagent of fatty acid to synthesize cellulose fatty ester. Gaofenzi Cailiao Kexue Yu Gongcheng 18, 11.

Sun, X.F., Xu, F., Sun, R.C., Fowler, P., Baird, M.S., 2005. Characteristics of degraded cellulose obtained from steam-exploded wheat straw. Carbohydrate Res. 340, 97–106.

Tebby, J.C., 1991. Handbook of Phosphorus-31 Nuclear Magnetic Resonance Data. CRC Press, Boca Raton, Florida.

Teymouri, F., Laureano-Perez, L., Alizadeh, H., Dale, B.E., 2005. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresour. Technol. 96 (18), 2014–2018.

Wadsworth Jr., W.S., Emmons, W.D., 1962. Bicyclic phosphites. J. Am. Chem. Soc. 84, 610–617.

Watanabe, Y., Komoda, Y., Ebisuya, K., Ozaki, S., 1990. An efficient phosphorylation method using a new phosphitylating agent, 2-(diethylamino)-1,3,2-benzodioxaphosphepane. Tet. Lett. 31 (2), 255–256.

Weil, J.R., Brewer, M., Hendrickson, R., Sarikaya, A., Ladisch, M.R., 1997. Continuous pH monitoring during pretreatment of yellow poplar wood sawdust by pressure cooking in water. Appl. Biochem. Biotechnol. 68, 21–40.

Wu, M.M., Chang, K., Gregg, D.J., Boussaid, A., Beatson, R.P., Saddler, J.N., 1999. Optimization of steam explosion to enhance hemicellulose recovery and enzymatic hydrolysis of cellulose in softwoods. Appl. Biochem. Biotechnol., 77–79.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y., 2005. Coordinated development of leading biomass pretreatment technologies. Bioresour. Technol. 96, 1959–1966.

Xiang, Q., Lee, Y.Y., Pettersson, P.O., Torget, R.W., 2003. Heterogeneous aspects of acid hydrolysis of α-cellulose. Appl. Biochem. Biotechnol. 105–108, 505–514.

Yang, K., Wang, Y.-J., Kuo, M.-I., 2004. Effects of substrate pretreatment and water activity on lipase-catalyzed cellulose acetylation in organic media. Biotechnol. Prog. 20, 1053.

Zhang, Y.P., Lynd, L.R., 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnol. Bioeng. 88, 797.

Zhu, S., Wu, Y., Chen, Q., Yu, Z., Wang, C., Jin, S., Ding, Y., Wu, G., 2006. Dissolution of cellulose with ionic liquids and its application: a mini-review. Green Chem. 8, 325.