

THE SUPERIORITY OF GAMMA-VALEROLACTONE (GVL) AS A NOVEL PRETREATMENT AGENT TO ENHANCE THE ENZYMATIC DIGESTIBILITY OF COTTON STALKS

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Abstract

Cotton stalks were subjected to pretreatments using GVL-H₂O, GVL-H₂O-H₂SO₄, GVL-TEAHSO₄, TEAHSO₄-H₂O and EMIMAc at 135°C and biomass loadings of 10% and 15% for one hour. The results demonstrated GVL as a promising pretreatment agent since GVL-H₂O pretreated biomass gave similar enzymatic hydrolysis yields compared to the yields obtained from those subjected to pretreatment via triethylammonium hydrogen sulfate (TEAHSO₄). GVL-H₂O (80 wt. %-20 wt. %) pretreated cotton stalks at 10% biomass loading resulted in 37.6% biomass digestibility at the 48th hour of the enzymatic reaction which was roughly 12-fold higher than the digestibility of untreated samples. It was also concluded that GVL-H₂O in the absence of an acid catalyst successfully deconstructed the cotton stalks at higher biomass loadings (15%) which hasn't been yet shown in the literature. The digestibility increased from 37.6% to 39.3% at the 48th hour of the enzymatic hydrolysis of cotton stalks subjected to GVL-H₂O pretreatment when biomass loading increased from 10% to 15%.

Key words: gamma-valerolactone, ionic liquid, pretreatment, lignocellulosic biomass, enzymatic hydrolysis

1. INTRODUCTION

Biomass deconstruction has a vital role in biomass processing to fuels and chemicals. Depending on the type of desired product(s), deconstruction strategies either bring in significant changes in the physical and chemical structure of lignocellulose or enable biomass fractionation. Dilute acid hydrolysis and steam explosion being useful for hemicellulose removal, alkaline pretreatment exploited for delignification and mechanical comminution that disrupt crystalline cellulose have been familiar strategies for years. Employment of ionic liquids for biomass deconstruction is relatively recent compared to the aforementioned techniques. Though ionic liquids (ILs) have been first explored as successful solvents in terms of cellulose dissolution and reducing the cellulose crystallinity (Swatloski *et al.*, 2002), the research shifted towards the use of ILs in biomass fractionation (Labbe *et al.*, 2012; da Costa Lopes *et al.*, 2013; Verdia *et al.*, 2014; An *et al.*, 2015; Peleteiro *et al.*, 2015; Yan *et al.*, 2015; Peng Zhang *et al.*, 2015) with the development of ILs capable of isolating high purity cellulose and lignin from the structure. For instance, Weigand *et al.* explored the low-cost IL, triethylammonium hydrogen sulfate (TEAHSO₄) to delignify and remove hemicellulose from willow. Pretreated biomass was converted to glucose with roughly 82% yield. Recently, Brandt-Talbot *et al.* showed that TEAHSO₄ solubilized more than 85% of lignin and hemicellulose present in the untreated Miscanthus and thereby, insoluble fraction of the biomass comprising mostly cellulose was converted into glucose with up to 77% yield (Brandt-Talbot *et al.*, 2017).

In the last decade, organosolv pretreatment of lignocellulosic biomass has received a different perception by the inclusion of biomass derived organic solvents (Z. Y. Zhang *et al.*, 2016). Selection of a suitable organic solvent for biomass deconstruction introduces several aspects into consideration. Along with the features associated with the energy expenses, efficiency and reuse of the solvent, one must also respect the solvent's environmental footprint. In this context, biomass derived methyltetrahydrofuran (2-MeTHF), tetrahydrofuran (THF), levulinic acid, and γ -valerolactone (GVL) are promising candidates that entail environmental benefits over their synthetic counterparts. Methyltetrahydrofuran (methyl-THF) which can be synthesized by catalytic hydrogenation of levulinic acid (organic acid derived from cellulose) or furfural (degradation product of hemicellulose) can be readily degraded under the sunlight in air in case of any spillage (Pace *et al.*, 2012). Likewise, GVL

obtained upon catalytic hydrogenation of levulinic acid has remarkably low vapor pressure with a boiling point of 207°C making it easy to handle. The fact that not reacting considerably with oxygen and water makes GVL reliable for large scale utilization and environment friendly (Horvath *et al.*, 2008; Xue *et al.*, 2016). Thereby, utilization of solvents obtained from renewable resources leading to the sustainable production of fuels and chemicals offers the use of ecofriendly solvents that supports the 7th principle of Green Chemistry (Pace *et al.*, 2012).

GVL is a renewable heterocyclic ester obtained from catalytic hydrogenation of levulinic acid which is an attractive platform chemical obtained from cellulose. (D. M. Alonso *et al.*, 2013). Several reports discussed the valorization of GVL as a platform chemical for the production of C₈₊ alkanes through successful catalytic routes (David Martin Alonso *et al.*, 2010; Bond *et al.*, 2010). Besides, GVL was employed as an efficient medium for the production of levulinic acid from fructose with yields ranging between 50-70% (Qi *et al.*, 2014) and the production of furfural from corncob with roughly 80% yield (L. X. Zhang *et al.*, 2014). The research reported by Luterbacher *et al.* (Luterbacher *et al.*, 2014) is an upswing in the field of lignocellulose conversion to fermentable sugars using GVL. Their success was attributed to the solubilization of carbohydrate fractions of different lignocellulosic feedstocks (corn stover, maple wood, loblolly pine) and recovery of soluble sugars from GVL to aqueous phase by simple addition of NaCl or liquid CO₂. Besides, Luterbacher *et al.* found that a solvent mixture containing 80 wt% GVL and 20 wt.% H₂O was able to extract lignin from corn stover through a reaction conducted at 20 wt.% biomass loading and 393 K for 30 min (Luterbacher *et al.*, 2015). The isolated lignin was structurally similar to native lignin. This finding was linked to the reaction conditions being less severe. In another study, 50-60 wt.% GVL solutions were employed for fractionation of Eucalyptus globulus (Le *et al.*, 2016) at 180°C for one hour. The composition of the resulting pulps indicated that GVL solutions enabled roughly 75% hemicellulose and 90% lignin removal and thus, original cellulose content of the biomass was preserved. Above all, the resulting pulp was directly converted into cellulose fibers possessing comparable mechanical properties to those demonstrated by the similar products currently in the market. Motagamwala *et al.* (2016) proposed a novel strategy by including an organic solvent to the GVL-H₂O-acid system. The proposed strategy provided much higher sugar yields and easier recovery for the principle biomass components compared to the previous findings reported by Luterbacher and his co-workers in 2014. Inclusion of organic solvent, toluene, resulted in the formation of two separate phases after the reaction was cooled to room temperature. While organic phase was rich in GVL, aqueous phase was rich in glucose, xylose, furfural and levulinic acid. Lignin was obtained as an insoluble fraction apart from organic aqueous phases. (Motagamwala *et al.*, 2016).

In this study, cotton stalks were pretreated with GVL-H₂O, GVL-H₂O-H₂SO₄ and GVL-TEAHSO₄ solutions to identify the performance of GVL as a novel pretreatment agent for the deconstruction of cotton stalks. Besides, TEAHSO₄-H₂O solution and EMIMAc were included as controls since their performances on biomass deconstruction have been demonstrated previously. All pretreatments were conducted at 10 wt.% and 15 wt.% biomass loadings to explore the capability of GVL to operate at higher biomass loadings. The enzymatic accessibility of untreated and pretreated cotton stalks was monitored in terms of biomass digestibility. Besides, lignin extraction was assessed to decide on the effectiveness of GVL on biomass fractionation.

2. MATERIALS AND METHODS

2.1 Chemicals

Cotton stalks were air-dried, ground and sieved to particle size less than 1 cm prior to pretreatments. Trisodium citrate dihydrate, citric acid monohydrate, potassium sodium tartrate tetrahydrate, sodium hydroxide, sulfuric acid, calcium carbonate, acetic acid, D-glucose were purchased from Merck (Darmstadt, Germany). Gamma-valerolactone, phenol, 3,5-dinitrosalicylic acid, sodium sulfite, triethylamine and 1-ethyl-3-methyl imidazolium acetate (EMIMAc) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cellulase enzyme, Cellic Ctec2 was kindly provided by Novozymes (Denmark).

2.2 Synthesis of TEAHSO₄ and preparation of pretreatment solutions

247 mmols of triethylamine was put in a round bottom flask and mixed with an equal volume of water. The round bottom flask, which was cooled by an ice bath, was placed on a magnetic stirrer. An aqueous solution of sulfuric acid was prepared by mixing 13.4 ml of 18.4 M sulfuric acid and 60 ml water. Sulfuric acid solution was added dropwise to trimethylamine solution under stirring. The ionic liquid solution was stirred overnight. The water from TEAHSO₄ was removed via rotary evaporator. The resultant product was a white hygroscopic solid.

GVL and TEAHSO₄ solutions were prepared according to the following proportions: GVL-H₂O (80 wt.% GVL-20 wt.% H₂O), GVL-H₂O-H₂SO₄ (80 wt.% GVL-20 wt.% H₂O-10 mM H₂SO₄), GVL-TEAHSO₄ (80 wt.% GVL-20 wt.% TEAHSO₄) and TEAHSO₄-H₂O (80 wt.% GVL-20 wt.% H₂O).

2.3 Pretreatments

A simplified flow chart presenting the details of pretreatments is given in Figure 1. Cotton stalks were dried at 105°C overnight prior to all pretreatments. Pretreatment of cotton stalks were conducted in Pyrex tubes with teflon-lined screw caps which were placed in an oven. In a typical pretreatment, 10 g of GVL-H₂O, GVL-H₂O-H₂SO₄, GVL-TEAHSO₄, TEAHSO₄-H₂O solutions and EMIMAc were mixed with the biomass and incubated at 135°C without stirring for one hour. Biomass loading was investigated at 10 and 15 wt.%. Following the pretreatments, the samples were allowed to cool to room temperature. Then, ethanol was added as an antisolvent and the solution was stirred for 20 minutes. The cellulose rich solid was separated by filtration and washed with ethanol three times. Finally, washed cellulose rich solid fraction was dried at 70°C under vacuum prior to its enzymatic saccharification and its weight was recorded to determine the recovery of cellulose rich solid or in other words, solid recovery (SR) as follows: $SR (\%) = (W_{\text{cellulose rich solid}} / W_{\text{untreated biomass}}) \times 100$.

Ethanol in the liquid fraction was removed by rotary evaporator at 50°C. Following the ethanol evaporation, water was added to precipitate lignin present in the [TEAH][SO₄] solution. Lignin was recovered by centrifugation at 6000 rpm for 15 minutes, washed with water three times and, finally dried at 70°C under vacuum. The weight of the dried lignin was recorded and the lignin precipitate yield (LPY) was calculated as follows: $LPY (\%) = (W_{\text{dried lignin precipitate}} / W_{\text{lignin in the untreated biomass}}) \times 100$.

The lignin content of the untreated and pretreated cotton stalk samples was determined based on the procedure of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). The percentage lignin extracted (%) was calculated according to the previously reported equation (N. I. Haykir *et al.*, 2013). All pretreatments were conducted in duplicate and the average values are reported.

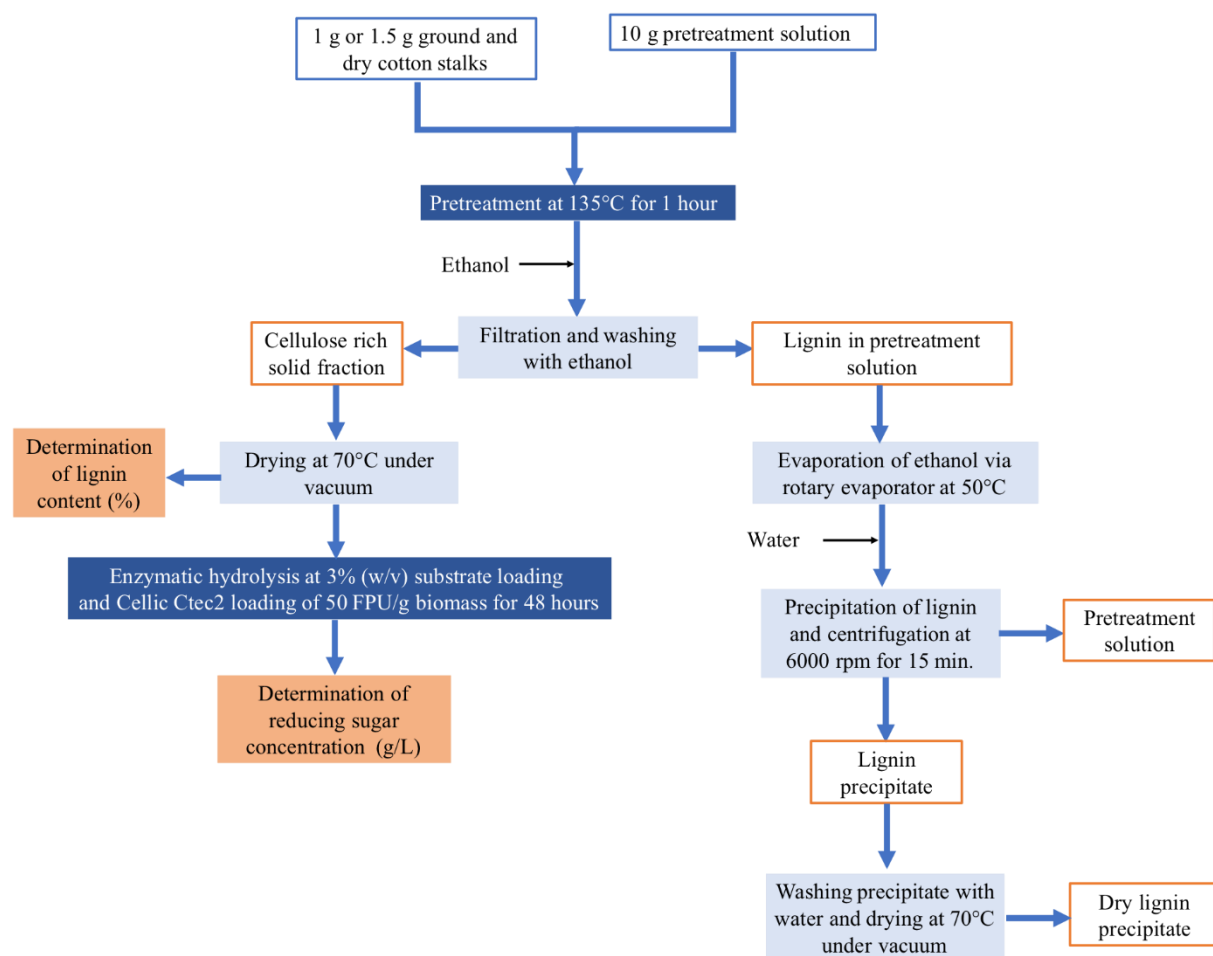


Figure 1. Simplified flowchart for the pretreatment of cotton stalks via various pretreatment agents.

2.4 Enzymatic hydrolysis

Enzymatic hydrolysis of untreated and pretreated cotton stalks was performed at 3% (w/v) biomass loading in 0.05 M sodium citrate buffer at pH 4.8 and 50°C in water bath. The enzyme, Cellic Ctec2 was used at a loading of 50 FPU/g biomass. The reaction was monitored for 48 h. At specific time intervals, 150 μ L of samples were withdrawn. The samples were incubated at 100°C for 5 min in order to stop the enzymatic reaction and finally centrifuged at 6000 rpm for 10 minutes prior to analysis. The reducing sugars released during the reaction were analyzed via DNS method (Miller, 1959) to determine the digestibility of cotton stalks which was calculated as follows: Biomass Digestibility (%) = $(C_R/C_B) \times SR(\%)$ where, C_R is the reducing sugar concentration in the enzymatic hydrolyzate (g/L), C_B is the initial concentration of the biomass in the hydrolysis buffer that is subjected to enzymatic hydrolysis (g/L) and SR (%) is the percentage solid recovery.

3. RESULTS AND DISCUSSION

3.1 Effect of pretreatment agents and biomass loadings on solid recovery and lignin extraction

Figure 2 shows the effect of pretreatment agents and biomass loadings on the solid recovery. According to the figure, the lowest solid recovery was attained for TEAHSO₄-H₂O pretreated cotton stalks in which almost half of the biomass was lost during the pretreatment. This result was attributed to the unique solubilization power of TEAHSO₄ towards lignin (George *et al.*, 2015; Brandt-Talbot *et al.*, 2017). As the biomass loading increased from 10% to 15% for all pretreatment agents, the solid recovery increased as expected. While 75.7% of solid is recovered after GVL-H₂O pretreatment at 10% biomass loading,

the solid recovery decreased to 65.7% with the addition of 10 mM sulfuric acid to the pretreatment agent. Dilute sulfuric acid has been well recognized to easily degrade the carbohydrate fractions of biomass at high temperatures (Yang and Wyman, 2008). On the other hand, GVL-TEAHSO₄, which hasn't been evaluated previously in terms of the agents' combined impact on biomass pretreatment, gave much lower solid recovery (63.9% at 15% biomass loading) than that obtained upon GVL-H₂O pretreatment. Apparently, TEAHSO₄ solubilized lignin in the cotton stalks even in combination with GVL.

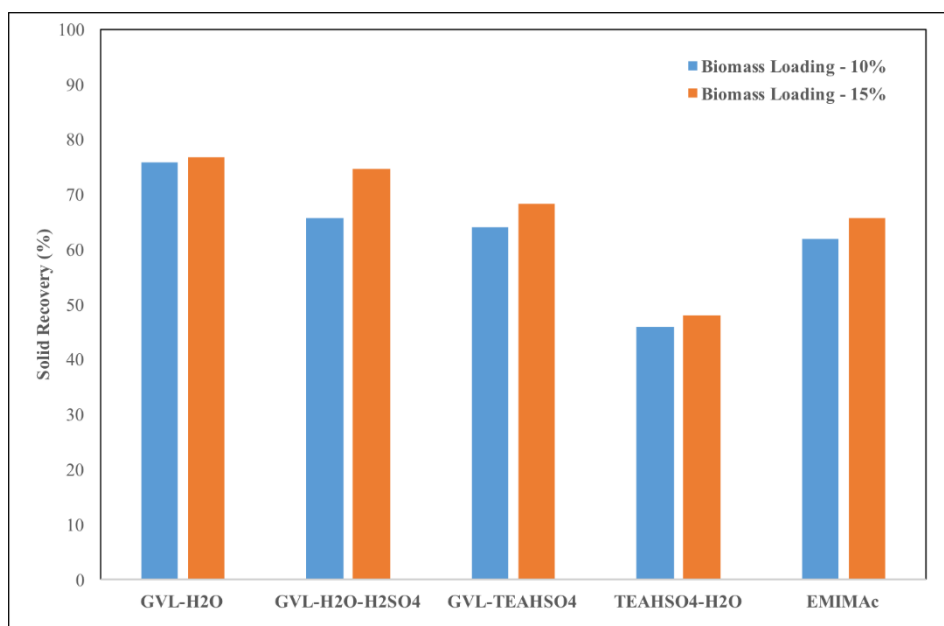


Figure 2. Effect of pretreatment agents and biomass loading on solid recovery (%).

As shown in Figure 3, lignin extraction ranged between 29% and 34% for the cotton stalks pretreated via GVL-H₂O, GVL-H₂O-H₂SO₄, GVL-TEAHSO₄ at 10% biomass loading. The highest lignin extraction was obtained as 56.1% using TEAHSO₄-H₂O for pretreatment at 10% biomass loading. This result was consistent with the solid recovery attained for cotton stalks subjected to TEAHSO₄-H₂O pretreatments. EMIMAc, which was not as efficient as TEAHSO₄-H₂O for removal of lignin, was only capable of removing 36.1% lignin at 10% biomass loading. As expected, lignin removal decreased with an increase in biomass loading for all pretreatment agents as shown in the figure.

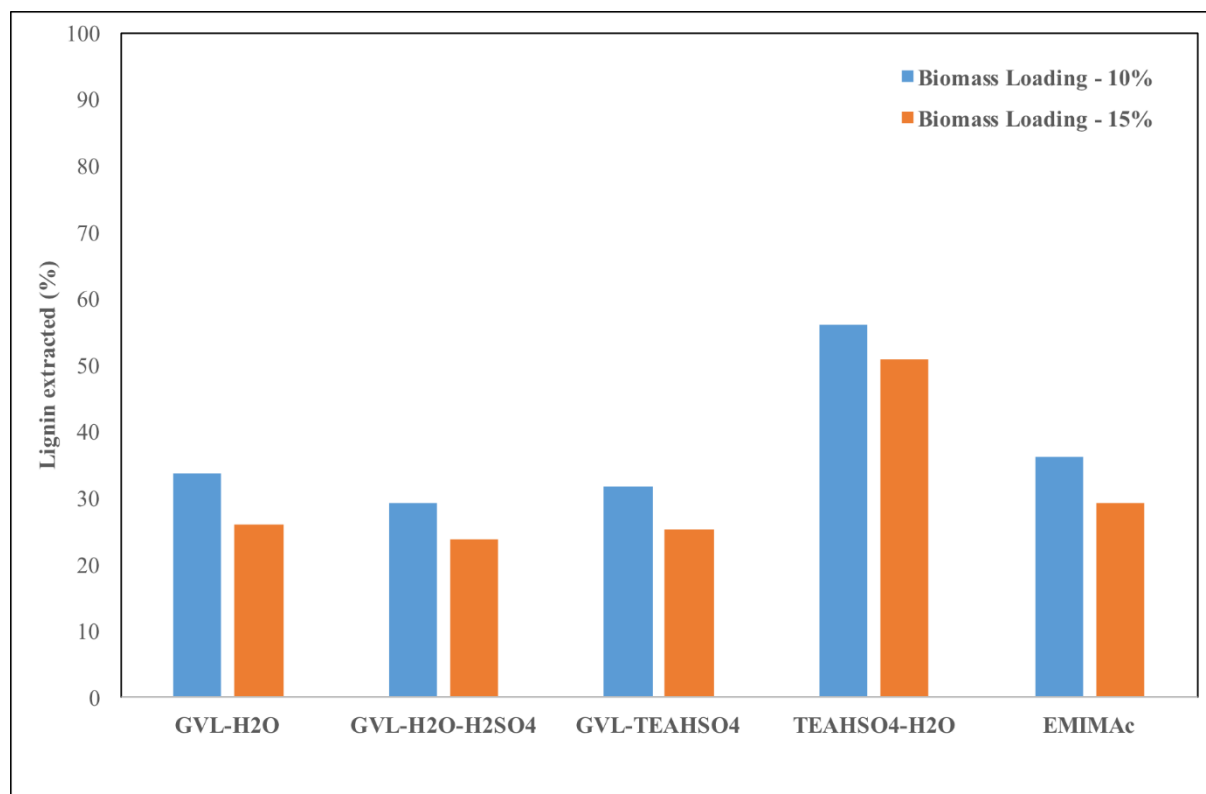


Figure 3. Effect of pretreatment agents and biomass loading on the lignin extracted from cotton stalks (%).

Figure 4 shows the physical appearance of cotton stalks before and after pretreatment. The physical structures of the biomass samples were almost retained except that pretreated via EMIMAc. EMIMAc pretreatment changed the appearance of the cotton stalks remarkably; the color of the biomass got darker and the fiber-like structure was no longer present in the samples. This significant change indicated the significant degree of biomass deconstruction exhibited by EMIMAc. EMIMAc may not only be capable of disrupting lignin, it may also degrade carbohydrate fractions in case of conducting the pretreatment at low biomass loadings and elevated temperatures. EMIMAc has been acknowledged as an excellent agent which can readily penetrate through recalcitrant lignocellulose and breakdown crystalline cellulose even at high biomass loadings (H. Wu *et al.*, 2011; Nazife Isik Haykir and Bakir, 2013). For the rest of the samples, yellow color of the untreated samples changed into brown while the original fibrous structure was retained.

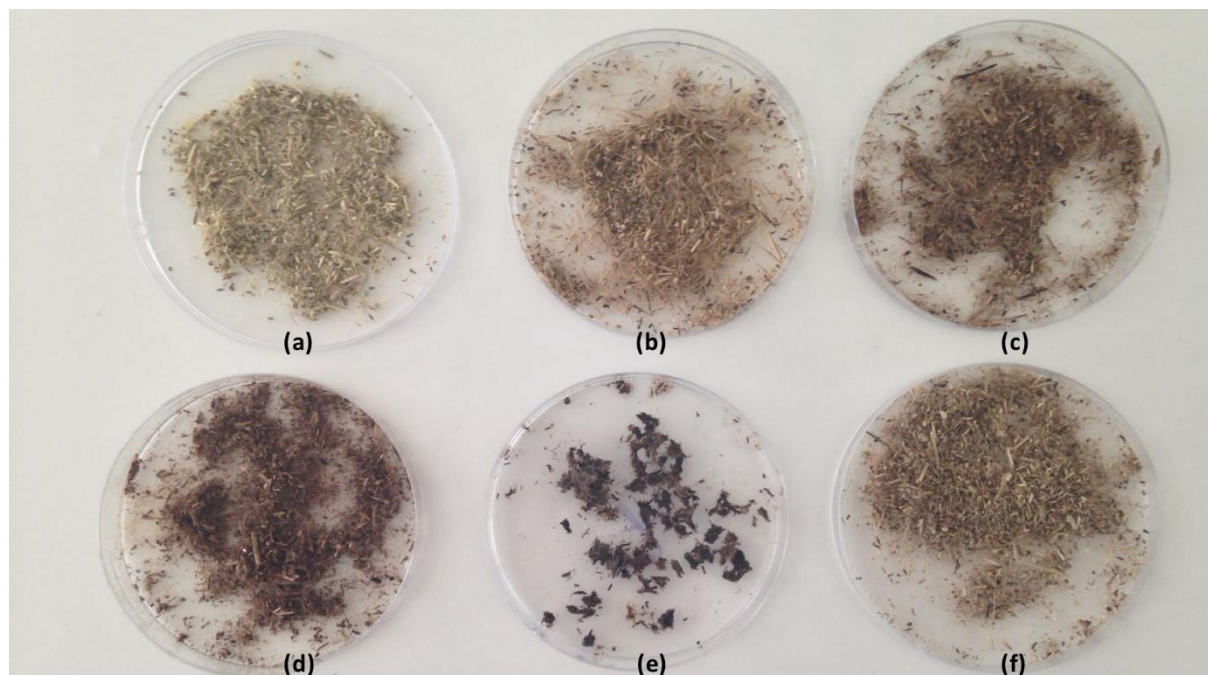


Figure 4. Physical appearance of untreated cotton stalks (a), pretreated cotton stalk samples via GVL-H₂O (b), GVL-TEAHSO₄ (c), TEAHSO₄-H₂O (d), EMIMAc (e) and GVL-H₂O-H₂SO₄ (f) at 10% biomass loading.

The effect of pretreatment agents and biomass loading on the yield of lignin precipitate (%) was also investigated (Figure 5). Following the pretreatments, lignin present in the pretreatment solution was precipitated by the addition of water as indicated in the previous part. The highest yield (76.1%) was obtained from TEAHSO₄-H₂O solution used for pretreatment at 10% biomass loading. Based on the amount of lignin extracted (56.1%), the lignin precipitate was expected to be around 50% following the TEAHSO₄-H₂O pretreatment. This difference might arise from the hemicellulosic fraction precipitated together with lignin. Similarly for the other samples, there is an inconsistency between the lignin extracted (%) and the lignin precipitate yield (%). Though the percentage lignin extracted, which was calculated based on the NREL procedure, ranged roughly between 23% and 34% for the samples pretreated via GVL solutions, lignin precipitate yield was obtained as 10% at most. This might be due to the method employed for lignin precipitation, which worked very well for TEAHSO₄-H₂O pretreatment, but wasn't suitable for lignin precipitation from the other pretreatment solutions.

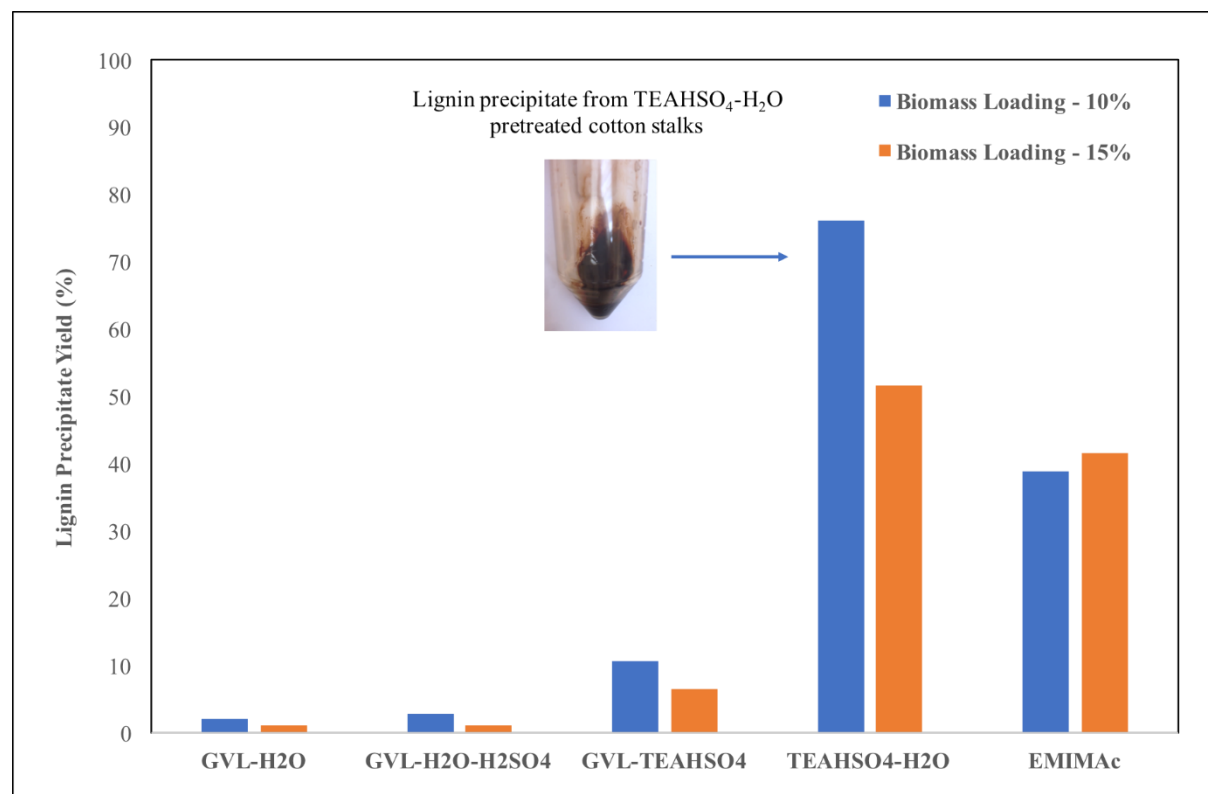


Figure 5. Effect of pretreatment agents and biomass loading on the lignin precipitate yield (%).

3.2 Effect of pretreatment agents and biomass loadings on biomass digestibility (%)

In this part, the enzymatic hydrolysis of untreated and pretreated cotton stalks was conducted for 48 hours with the aim to investigate the enhancement in the digestibility of pretreated samples with respect to untreated cotton stalks. The digestibility of the samples at the 48th hour of the enzymatic reaction is given in Figure 6. Recalling the deconstruction power of EMIMAc towards any lignocellulosic biomass, the results obtained for EMIMAc pretreated biomass was not surprising. Biomass digestibility was as high as 87% for the cotton stalks subjected to EMIMAc pretreatment. Among GVL pretreated samples, the highest digestibility was achieved as 41.7% for the cotton stalks pretreated via GVL-H₂O-H₂SO₄ at 15% biomass loading. The combination of GVL and TEAHSO₄ did not enhance enzymatic accessibility of biomass significantly; 37.2% of biomass digestibility was achieved for the samples pretreated at 15% loading. Interestingly, GVL-H₂O pretreated biomass at 10% loading yielded very close digestibility to that obtained upon enzymatic hydrolysis of TEAHSO₄-H₂O pretreated cotton stalks. They were 37.6% and 38.6%, respectively. Considering the pretreatments conducted at 15% loading, GVL-H₂O pretreatment gave much higher digestibility (39.3%) compared to that pretreated via TEAHSO₄-H₂O (32.5%). Despite the fact that the performance of GVL-H₂O in lignin extraction was quite poor compared to TEAHSO₄-H₂O, GVL did really well considering its impact on biomass enzymatic accessibility.

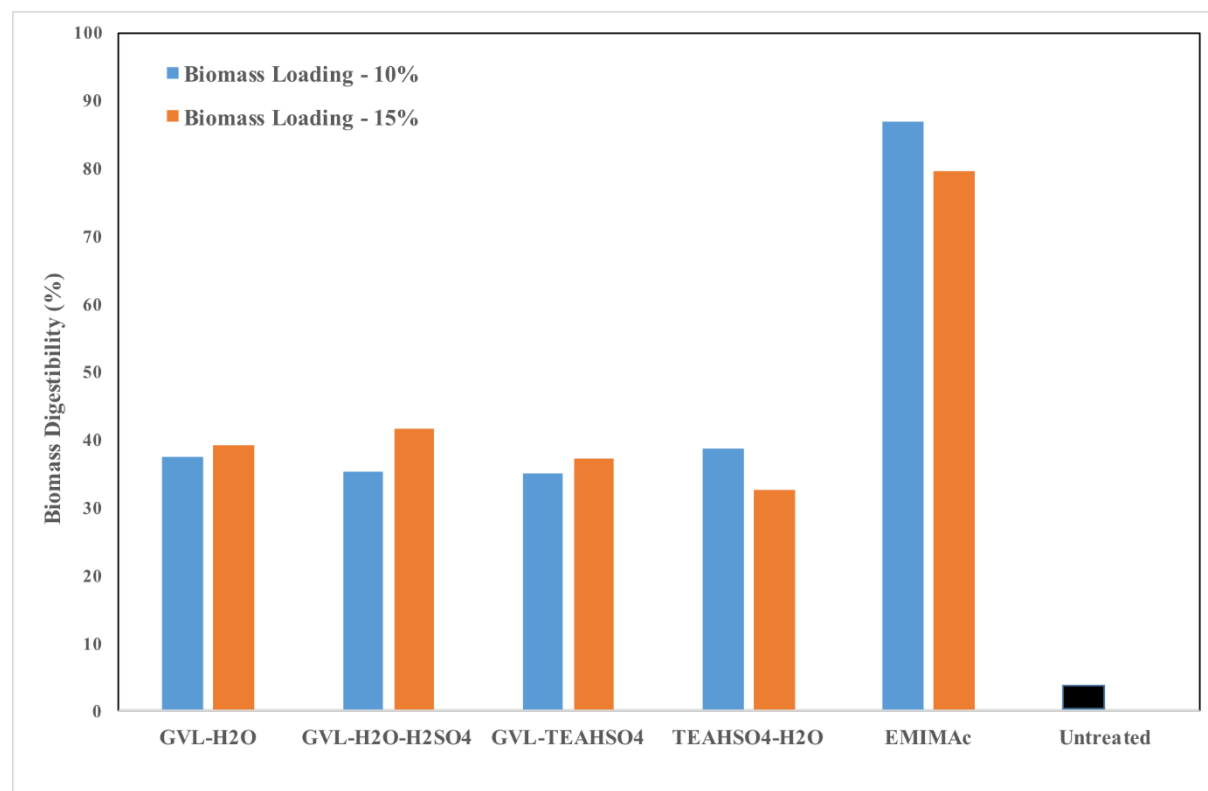


Figure 6. Effect of pretreatment agents and biomass loading on the biomass digestibility (%).

Considering the mild pretreatment conditions at which GVL-H₂O pretreatment was conducted together with the enhancement in the biomass digestibility with GVL-H₂O pretreatment, GVL should be regarded as an effective pretreatment agent. Pretreatment of biomass using GVL in the absence of an acid catalyst at a temperature as low as 135°C and biomass loading of 15% has not been attempted previously. It has been shown in many of the studies that GVL was employed at temperatures between 160°C and 180°C and biomass loadings roughly between 6 wt.% and 10 wt.% (Luterbacher *et al.*, 2014; Le *et al.*, 2016; Motagamwala *et al.*, 2016; M. Wu *et al.*, 2016). An exception is the study reported by Shuai *et al.* in which pretreatment of beech wood chips by GVL-H₂O (80%-20%) was conducted at 120°C and biomass loading up to 20% in the presence of 75 mM sulfuric acid. They obtained lignin removal up to 80% and almost complete retention of cellulose in the original biomass. Accordingly, the enzymatic hydrolysis of the pretreated biomass gave 85-100% sugar yield (Shuai *et al.*, 2016). A similar work was performed by Wu *et al.* in which cotton stalk powder was pretreated via GVL-H₂O at different proportions at a solid to liquor ratio of 1:15 g/mL and 170°C for 1 h in the presence of 10 mM H₂SO₄ (M. Wu *et al.*, 2016). They used a different strategy compared to the procedure used in this study for separation of lignin; addition of NaCl to the pretreatment slurry resulted in the formation of a biphasic solution. Almost 93% of glucose in the biomass was yielded and 10%-15% lignin removal was achieved.

4. CONCLUSIONS

In this study, biomass deconstruction via GVL was conducted in different conditions. Pretreatment conducted at milder conditions (at 135°C and 15% biomass loading for 1 hour) introduced significant changes in the digestibility of cotton stalks; biomass digestibility of 39.3% was obtained at the 48th hour of the enzymatic hydrolysis. Aqueous GVL solution readily pretreated cotton stalks at a particle size less than 1 cm without any requirement of an acid catalyst at the specified conditions. These major findings promise the use of GVL as an effective pretreatment agent for the deconstruction of a variety of lignocellulosic feedstocks in the future.

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