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Pretreatment of Switchgrass for Production of Glucose via Sulfonic Acid-Impregnated Activated Carbon

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Abstract: In the present research, activated carbon-supported sulfonic acid catalysts were synthesized and tested as pretreatment agents for the conversion of switchgrass into glucose. The catalysts were synthesized by reacting sulfuric acid, methanesulfonic acid, and *p*-toluenesulfonic acid with activated carbon. The characterization of catalysts suggested an increase in surface acidities, while surface area and pore volumes decreased because of sulfonation. Batch experiments were performed in 125 mL serum bottles to investigate the effects of temperature (30, 60, and 90 °C), reaction time (90 and 120 min) on the yields of glucose. Enzymatic hydrolysis of pretreated switchgrass using Ctec2 yielded up to 57.13% glucose. Durability tests indicated that sulfonic solid-impregnated carbon catalysts were able to maintain activity even after three cycles. From the results obtained, the solid acid catalysts appear to serve as effective pretreatment agents and can potentially reduce the use of conventional liquid acids and bases in biomass-into-biofuel production.

Keywords: activated carbon; sulfonic solid; catalyst; pretreatment; switchgrass; Ctec2; glucose

1. Introduction

There has been significant interest in using lignocellulosic biomasses as feedstock to produce fuels and chemicals [1–3]. Specifically, switchgrass has attracted the attention of bioenergy researchers due to its faster rate of growth, even on non-arable lands with limited agronomic inputs [4–7]. With yields ranging between 13.5 and 17.9 Mg ha^{-1} and an ethanol production potential of 60 GJ ha^{-1} yr^{-1}, switchgrass is an excellent choice as a biofuel feedstock [8,9].

The conversion of switchgrass to alcohols involves the pretreatment of switchgrass, followed by hydrolysis and fermentation [10–14]. Pretreatment is an important step in the switchgrass-to-alcohol and chemical supply chain [1,15]. Pretreatment allows for disruption of the switchgrass structure [16,17] by breaking down the lignin that binds to cellulose and hemicelluloses, reducing the crystalline structure of cellulose and increasing the available surface area that facilitates enzymatic reactions with cellulose and hemicelluloses [18,19]. Hence, several pretreatment methods, viz., acid [20,21], base [22,23], ammonia [24,25], hot water [19], and ozone [26,27], have been explored extensively for the last two decades [16,18,28]. However, dilute sulfuric acid pretreatment (140–215 °C) is most used [29]. Sulfuric acid works well as a pretreatment agent by solubilizing hemicellulose and depolymerizing lignin [21]. However, sulfuric acid is highly corrosive and requires specialized equipment to pretreat biomass [16]. Moreover, the spent liquor needs additional downstream treatment before its safe disposal, thereby adding costs to the overall process.

One approach to minimizing the corrosion and downstream treatment problems associated with sulfuric acid pretreatment is to employ recyclable solid acid catalysts [28,30,31]. Solid acid catalysts are simple to synthesize and can be reused several times with minimal loss in activity [32–34]. Nonetheless, based on the review of the

literature, few studies have been performed on the solid acid catalyst pretreatment of biomasses. Pena et al. [35] synthesized propyl sulfonic groups on silica-coated cobalt-iron oxide nanoparticles to serve as solid acid catalysts for the pretreatment of corn stover (160–200 °C and 60 min). When the pretreated corn stover was hydrolyzed via Accellerase, an average glucose yield of 59% was observed. Similarly, Dowex (TM) Dr-G8 was systematically employed as a solid catalyst by Tan and Lee [36] for the treatment of microalgal residue to study the effects of catalyst loading, pretreatment time and temperature, and biomass loading. When pretreated algal residue was hydrolyzed, glucose yields up to 99% were recorded under optimal conditions (4% catalyst loading, 120 °C, 30 min pretreatment time, and 30 h hydrolysis at 45 FPU). Furthermore, the catalyst was able to maintain activities for up to five cycles. As suggested in these reports, solid acid catalysts offer great potential as pretreatment agents. Similarly, Qi et al. [37] evaluated a solid acid carbon catalyst derived from cellulose-impregnated sulfuric acid for pretreatment of corncob and reported that sulfonic group-supported carbon catalysts were effective in pretreating corn cob powder with an enzymatic digestibility between 91% and 96% while maintaining activity after five reuses.

Considering the importance of switchgrass as a bioenergy crop in the U.S. and across the world, the next step would be to develop solid-acid catalysts that can pretreat switchgrass effectively and at lower temperatures than conventional liquid sulfuric acid. In this regard, sulfonic acid-supported carbons offer great promise based on their high activity and selectivity in several acid-catalyzed reactions [38–42]. However, for scalability purposes, the choice of sulfonic acid precursor plays an important role. Currently, however, there is not much information on the efficacy of different sulfonic acids, especially for switchgrass processing. Therefore, the overall goal of this research is to synthesize and systematically compare different sulfonic solid acid-impregnated carbon catalysts side-by-side for pretreating switchgrass for subsequent hydrolysis into glucose. Sulfonic acid catalysts are usually synthesized via a reaction between sulfuric acid and carbon supports. In addition, methanesulfonic acid (MSA) and *p*-toluene sulfonic acid (pTSA) have also been investigated as environmentally friendly acids for the processing of biomasses [43,44]. Supported MSA was shown to be significantly less corrosive than sulfuric acid, biodegradable relative to other mineral acids, and an effective acid catalyst for conversion of glucose into levulinic acid and for fatty acid esterification reactions [44,45]. Similarly, pTSA was reported to be more stable and active than sulfuric acid based sulfonic acid catalysts in esterification reactions with succinic acid containing ethanol [46]. Thus, the specific objectives focused on: (1) synthesizing activated carbon (AC)-impregnated sulfonic acid catalysts using sulfuric acid (SA), *p*-toluene sulfonic acid (pTSA), and methanesulfonic acid (MSA) as precursors; (2) testing the effects of pretreatment time and temperature on glucose yield; and (3) evaluating the reusability of the acid catalysts in pretreatment reactions.

2. Materials and Methods

2.1. Switchgrass Preparation

Alamo switchgrass used in this study was harvested from North Carolina State University Field Laboratory in Reedy Creek Road Field, Raleigh, NC. Switchgrass was dried in the field for 3 days and baled with a conventional square hay baler. Switchgrass samples were then ground to pass a 2 mm sieve, transferred into air-tight plastic bags, and stored at room temperature until they were used.

2.2. Catalyst Preparation

Three solid acid catalysts were synthesized using activated carbon and *p*-toluenesulfonic acid (pTSA), methanesulfonic acid (MSA), and sulfuric acid (SA) as precursors. Briefly, 50 g of activated carbon (2–3 mm granules; C270C, Fisher Scientific) was impregnated with 100 mL of MSA and SA for 6 h. For the pTSA catalyst, the support (activated carbon) was impregnated with a solution of pTSA in water (67 g dissolved in 100 mL water), followed by washing with deionized (DI) water (1 h) and re-soaking in DI

water overnight. Subsequently, the catalysts designated as AC-SA, AC-MSA, and AC-pTSA to represent activated carbon impregnated with sulfuric acid, activated carbon impregnated with methanesulfonic acid, and activated carbon impregnated with *p*-toluenesulfonic acid, respectively, were dried at 105 °C (2 h), calcined at 250 °C (2 h), and stored in air-tight glass containers until further use.

2.3. Catalyst Characterization

2.3.1. BET Surface Area Analysis

The Gemini VII 2390 Brunauer–Emmett–Teller (BET) surface area analyzer from Micromeritics was used for the surface area analysis. The catalyst samples (approximately 0.5 g) were degassed at 150 °C (2 h) followed by nitrogen adsorption–desorption to determine the specific surface area, pore volume, pore size, and isotherms.

2.3.2. Determination of Surface Functional Groups

The Boehm titration method was used to quantify the surface functional groups of catalysts. Briefly, 0.5 g of catalyst was mixed with solutions of 0.05 M $NaHCO_3$, 0.05 M Na_2CO_3, and 0.05 M NaOH at 125 rpm for 24 h at room temperature as described by [47]. After separating the catalysts from the solutions, 10 mL of each solution was titrated with 0.05 M HCl using methyl red as an indicator. As suggested by [48], it was assumed that NaOH neutralized sulfonic, carboxylic, lactonic, and phenolic groups; $NaHCO_3$ neutralized sulfonic and carboxylic groups; and Na_2CO_3 neutralized sulfonic, carboxylic, and lactone groups.

2.3.3. Thermogravimetric Analysis

The catalyst samples were also analyzed using a thermogravimetric analyzer (TGA, Q500, TA Instruments, New Castle, DE, USA). Approximately 18–35 mg of catalyst sample was placed on a platinum pan and heated from 0 to 600 °C at a rate of 30 °C min^{-1} in a nitrogen atmosphere.

2.3.4. Energy-Dispersive Spectroscopy

A variable-pressure scanning electron microscope (Hitachi S3200) equipped with an Oxford energy-dispersive X-ray spectrometer was employed to obtain the elemental composition of the catalyst surface. Typically, the catalyst samples were placed on an adhesive carbon stub and the data was acquired via a beam energy of 0–20 keV.

2.3.5. X-ray Photoelectron Spectroscopy

Two to three randomly selected catalyst granules were mounted on a double-sided conductive sticky tab on a sample holder. The data were acquired via a SPECS FlexMod XPS equipped with Mg Kα excitation (1254 eV). The energy calibration was performed based on adventitious carbon that was referenced at 285.0 eV. Subsequently, the raw data were analyzed using Casa XPS software (Casa Software Limited, Devon, UK).

2.4. Catalytic Pretreatment of Switchgrass

Catalyst granules (1.5 g) and switchgrass (6 g) were mixed (350 rpm) with 90 mL of deionized water in a heated conical flask at atmospheric pressure. Temperatures of 30, 60, and 90 °C, and pretreatment times of 90 and 120 min, were selected. All experiments were performed in triplicates using a factorial experimental design. After pretreatment, the catalyst granules were manually separated, and biomass was vacuum-filtered. Subsequently, the catalyst was dried at 105 °C for 2 h and stored for subsequent reuse, while the recovered switchgrass was stored at 4 °C for subsequent enzymatic hydrolysis.

2.5. Reusability of the Catalyst

The reusability of the catalysts was assessed under all conditions. After the first use, the catalyst was separated and dried for 2 h at 105 °C and prepared for the next batch of

pretreatment. Pretreated switchgrass samples were separated and stored for enzymatic hydrolysis and composition analysis.

2.6. Enzymatic Hydrolysis

All pretreated switchgrass samples were hydrolyzed by mixing (150 rpm for 72 h) 1 g of switchgrass (dry basis) with 0.167 mL of Cellic® Ctec2 (19.8 FPU/g-solid biomass) (Novozymes North America, Franklinton, NC, USA) ((3.5% w/w (g enzyme protein g^{-1} dry biomass); activity $\approx$ 119 FPU mL^{-1}) [49] and 40 µg ml^{-1} of tetracycline hydrochloride (to minimize any bacterial growth during hydrolysis). Moreover, 0.05 M sodium citrate buffer was added to bring the total hydrolysate volume to 20 mL, corresponding with 5% solid loading.

2.7. Composition Analysis

The composition of raw and pretreated switchgrass was determined using standard National Renewable Energy Laboratory (NREL) procedures [50]. The samples were analyzed for acid-insoluble lignin (AIL), acid-soluble lignin (ASL), moisture, and carbohydrate contents (glucan, xylan, arabinan) (Table 1). AIL and ASL were determined via two-step acid hydrolysis in which switchgrass was hydrolyzed in 72% sulfuric acid at 30 °C for 1 h, followed by 1 h hydrolysis in 4% sulfuric acid at 121 °C. The clear acid hydrolysate was separated from solid residues via filtration through the crucible and stored at 4 °C for further analysis to determine ASL and total carbohydrate content via a UV–Vis spectrophotometer that was set to 205 nm. The retained solid residues were placed in an oven at 105 °C before being placed in a furnace at 550 °C for AIL determination. Total sugars including glucose, xylose, and arabinose were determined using high-performance liquid chromatography (HPLC) (Dionex UltiMate 3000, Dionex Corporation, Sunnyvale, CA, USA) equipped with a refractive index detector and an Aminex HPX-87H column set to 65 °C with an eluant (5 mM sulfuric acid) flow of 0.6 mL min^{-1}. The data were quantified based on comparison with glucose, xylose, and arabinose standards analyzed by the HPLC, and the glucose yields were calculated based on the remaining glucan content in the pretreated switchgrass samples.

Table 1. Compositional analysis of the raw switchgrass (SG) (control) and pretreated switchgrass with various sulfonic acid catalysts.

No	Composition	Raw Switchgrass	SA-Treated Switchgrass	MSA-Treated Switchgrass	pTSA-Treated Switchgrass
		Weight Percentage (%)	Weight Percentage (%)	Weight Percentage (%)	Weight Percentage (%)
1	Glucan	33.36 ± 1.02	29.15 ± 2.03	26.35 ± 1.08	26.22 ± 0.34
2	Xylan	20.84 ± 1.88	18.58 ± 1.28	15.78 ± 0.75	16. 54 ± 1.02
3	Arabinan	3.78 ± 1.1	2.88 ± 0.9	3.02 ± 0.34	2. 95 ± 0.8
4	Ash	2.65 ± 0.9	2.04 ± 0.04	1.95 ± 0.1	1.88 ± 0.43
5	Acid-Soluble Lignin	3.46 ± 0.75	2.86 ± 0.09	2.58 ± 0.07	2.46 ± 0.09
6	Acid-Insoluble Lignin	22.78 ± 0.4	20.62 ± 0.96	18. 84 ± 0.81	18.98 ± 0.93
7	Others	13.13 ± 0.3	Some parts lost during treatment. Solid Recovery less than 100%		
	Total	100%			

SA: Sulfuric acid; MSA: Methanesulfonic acid; pTSA: p-toluene sulfonic acid.

2.8. Experimental Design and Statistical Analysis

All experiments in this study were performed in triplicates and all catalysts were reused three times. Four treatment variables (catalyst type, pretreatment temperature, pretreatment time, and catalyst durability) were tested in this research. While catalyst (AC-SA, AC-pTSA, and AC- MSA), temperature (30, 60, and 90 °C), and catalyst durability (Run 1, Run 2, Run 3) had 3 levels, the pretreatment time (90 and 120 min) had 2 levels. A Proc Mixed model was used to analyze the data and a slice effects test was adapted to observe individual class/main and interaction effects for all treatment combinations using SAS 9.3 (Cary, NC) within 95% confidence limits using Type 3 tests of fixed effects.

3. Results and Discussion

3.1. Catalyst Characterization

The physicochemical analyses of the activated carbons are presented in Table 2. It appeared that after sulfonation treatment, the specific surface area and pore volume of all three sulfonic acid-supported carbon catalysts decreased (when compared with control) despite a constant pore diameter of around 20 Å. The surface area decreased, probably due to the oxidation reaction between carbon and sulfonic acid.

Table 2. Physicochemical characterization of the sulfonic acid catalysts used for pretreatment of switchgrass.

Characteristic	Raw AC	AC-SA	AC-MSA	AC-pTSA
Surface Area (m^2/g)	781 ± 31	734.5 ± 29	668.9 ± 26	317.8 ± 7
Pore Volume (cm^3/g)	0.4 ± 0.02	0.37 ± 0.03	0.33 ± 0.02	0.14 ± 0.00
Pore Size (Å)	20.75 ± 0.8	20.24 ± 0.8	20.23 ± 0.8	20.6 ± 0.4
Total Acidity (mmol g^{-1})	0.1	0.365	0.3	0.425
Carboxylic and Sulfonic (mmol g^{-1})	0.025	0.2	0.2	0.175
Lactone (mmol g^{-1})	0.025	0.09	0.05	0.125
Phenolic (mmol g^{-1})	0.05	0.075	0.05	0.125

Raw AC: Untreated activated carbon; AC-SA: Activated carbon treated with sulfuric acid; AC-MSA: Activated carbon treated with methanesulfonic acid; AC-pTSA: Activated carbon treated with p-toluene sulfonic acid.

Impregnation of sulfonic groups within the activated carbon framework reduced the available pore volume, which was also corroborated via adsorption isotherm plots (Figure 1). Our results are similar to the data presented by Liu et al. [51], who investigated the sulfonation of activated carbon and reported that surface area, pore volume, and pore diameter were reduced as a result of sulfonation, even though the pore diameter was consistent at around 20 Å in our research.

The data also suggest that the surface chemistry of activated carbon was altered substantially. The total acidity of the sulfonic acid-impregnated carbon catalysts increased substantially when compared to control (raw activated carbon). Specifically, the total acidity increased from 0.142 mmol g^{-1} (raw activated carbon) to 0.3, 0.365, and 0.42 mmol g^{-1} for AC-MSA, AC-SA, and AC-pTSA consisting of sulfonic, carboxylic, lactonic, and phenolic groups, indicating an oxidative reaction between activated carbon and sulfonic acid precursors. Further, when analyzed for the elemental analysis, sulfonic acid-supported carbons also exhibited a clear change in surface. The EDX spectra of raw AC and AC-MSA are presented in Figure 2. The surface exhibited the presence of sulfur at 2.3 keV due to the formation of sulfonic acid. Similar observations were reported by Shu et al. [52], who tested sulfonated asphaltic carbon catalyst in esterification reactions in which sulfur peaks were detected around 2.3 keV. Our characterization data were also corroborated via XPS analysis, which provided information about the surface chemistry of the sulfonated carbon. A comparison of XPS spectra obtained from raw AC and AC-MSA is shown in Figure 3. The XPS data obtained for untreated activated carbon indicated two distinct peaks of C 1s and O 1s, as well as iodine (perhaps from the manufacturer's chemical activation of activated

carbon) on the surface. However, the carbon impregnated with sulfonic acids exhibited peaks of C 1s, O 1s, S 2p, and iodine. The S 2p peak captured at 168.2 eV is indicative of sulfonic acid groups, as previously observed [39,42,53–55]. Further, the oxygen-to-carbon (O/C) ratio increased by 31% (from 0.12 to 0.158), suggesting the formation of surface oxygen groups, including sulfonic carboxylic and other acidic oxygen moieties capable of pretreating switchgrass.
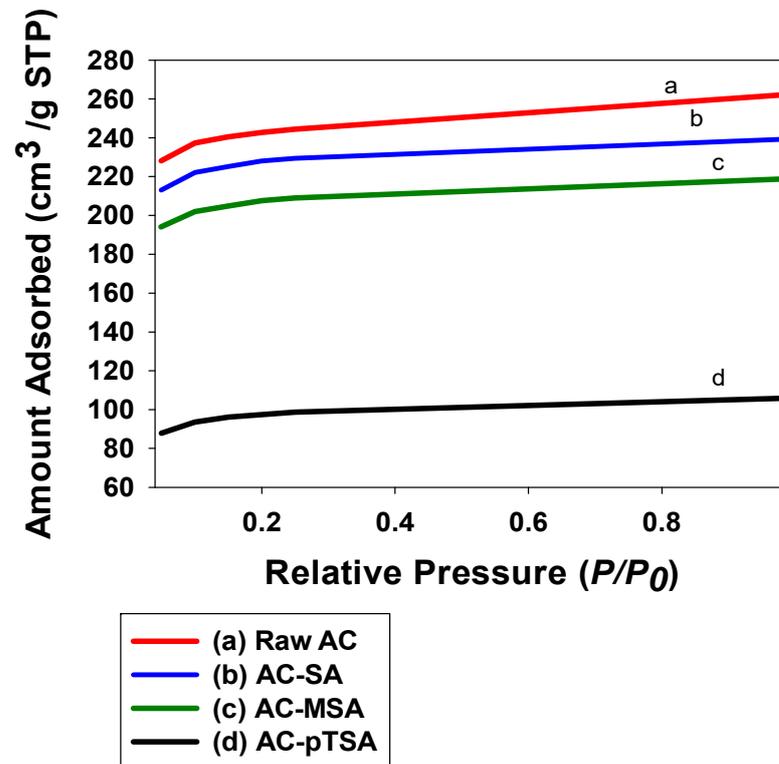

Figure 1. Adsorption isotherms of raw activated carbon (AC) (control) and sulfonic acid-impregnated AC catalysts.

The thermal stabilities of the raw and sulfonated catalysts were examined by thermogravimetric analysis (TGA) under a nitrogen gas atmosphere. As shown in Figure 4, the initial degradation of all samples was due to the presence of moisture or water adsorbed on the surface of activated carbon. The degradation profile of raw activated carbon (Figure 4, curve a) shows only one degradation peak that was observed before 100 °C and stayed considerably stable up to 600 °C, which was probably due to the evaporation of water molecules. Meanwhile, for sulfonic acid-impregnated ACs, two weight-loss results were observed. Overall, the first degradation was assumed to occur due to the evaporation of water adsorbed at the surface of catalysts [56]. The evaporation profiles in Figure 4 (curves b–d) suggest that the amount of water present in sulfonated catalysts was lower compared to the raw activated carbon, and therefore weight loss percentages for sulfonated acid catalysts were found to be lower compared to those for raw activated carbon. The second weight loss shown in Figure 4 (curves b–d) indicates that the sulfonated catalyst exhibited thermal stability up to 250–300 °C, as also observed by Shu et al. [52]. Beyond about 300 °C, desorption and decomposition of sulfonic groups (SO_3H) may have occurred, as suggested by Alvear-Daza et al. [57].
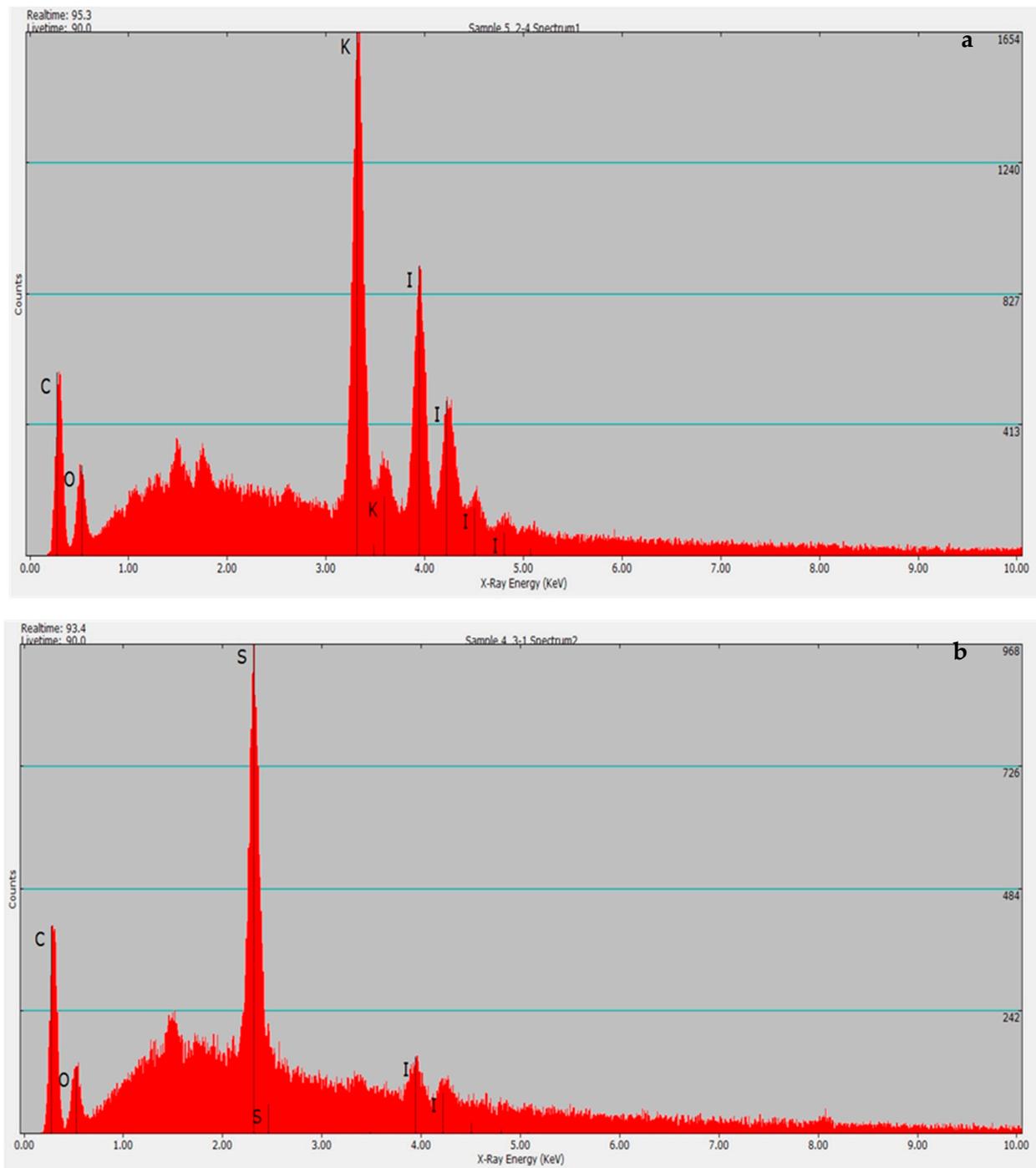
Figure 2. Energy-dispersive X-ray spectra obtained from raw activated carbon (**a**) and methanesulfonic acid (MSA)-impregnated AC (**b**).
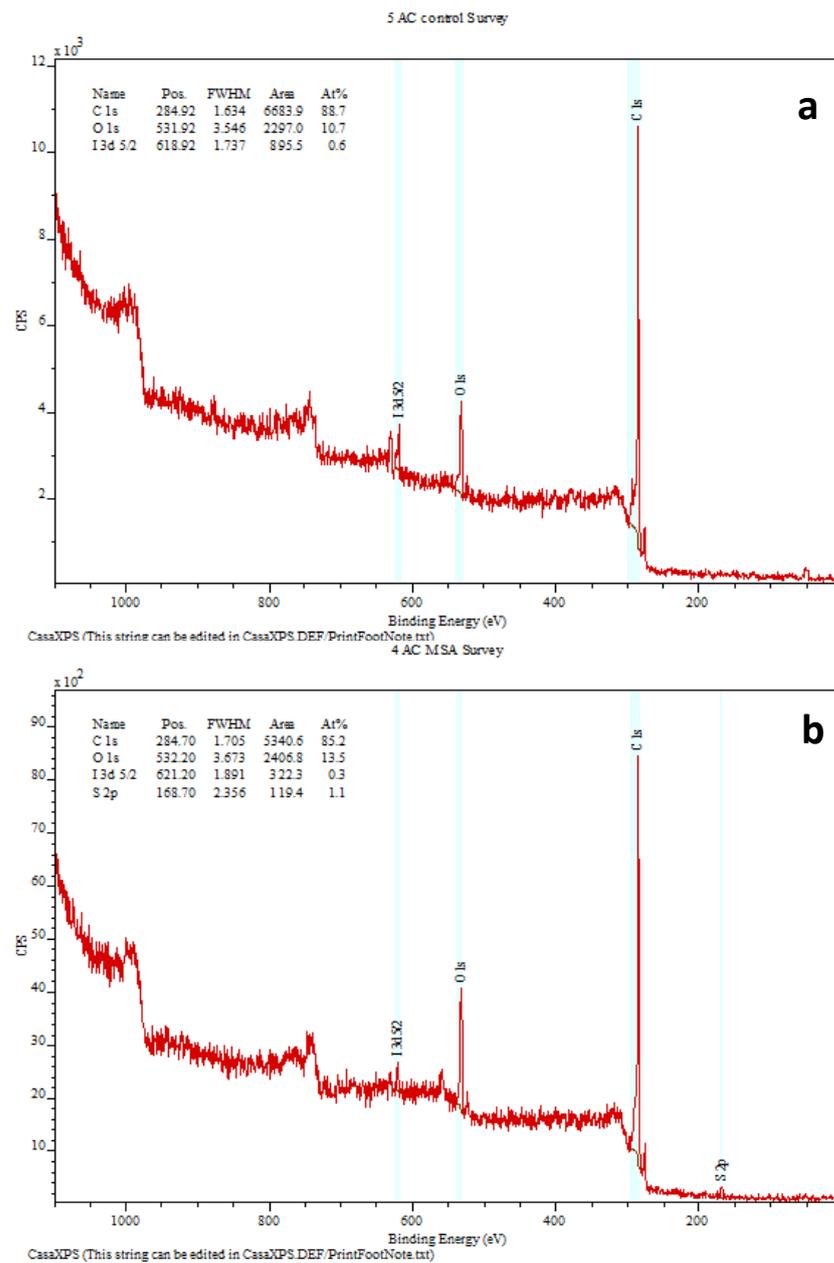
Figure 3. X-ray photoelectron spectra obtained from raw activated carbon (**a**) and MSA-impregnated AC (**b**).
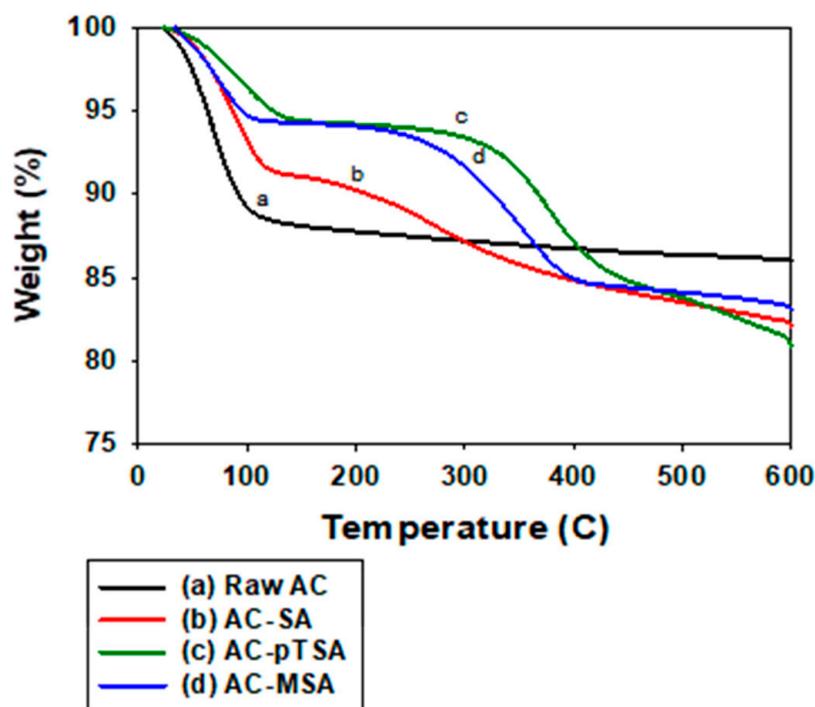
Figure 4. TGA data (weight loss percentage (%) vs temperature (°C)) for raw and MSA-impregnated AC.

3.2. Effect of Pretreatment Temperature on Glucose Production
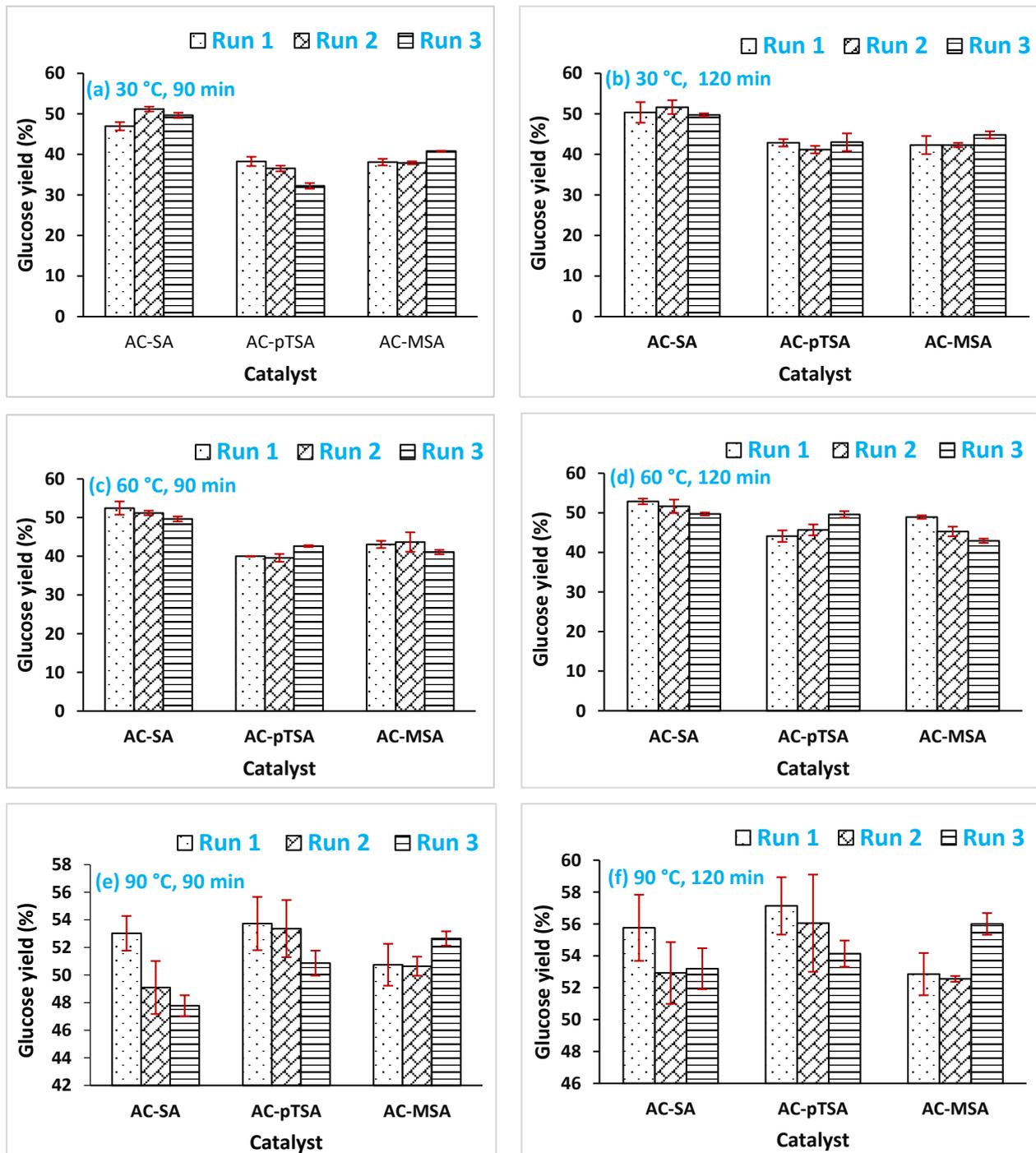
The effect of temperature on glucose yield for all catalysts is presented in Figure 5. The temperature was found to have a significant effect on glucose yields ($p < 0.05$) obtained from switchgrass treated with three different catalysts: AC-SA, AC-pTSA, and AC-MSA. For the temperature range tested (30–90 °C), glucose yield ranged from 31.5% to 57.13% for AC-pTSA, from 37.91% to 56.01% for AC-MSA, and from 46.96% to 55.75% for AC-SA.

Interestingly, AC-SA provided higher yields even at lower temperatures (30 and 60 °C) when compared to AC-pTSA and AC-MSA for both 90 and 120 min pretreatment times. However, as the temperatures were increased to 90 °C, no difference in glucose yields was observed for all catalysts tested ($p = 0.05$ based on type 3 tests of fixed effects. These data suggest that AC-pTSA and AC-MSA are activated at higher temperatures and likely enhance the rate of reaction between switchgrass and sulfonic acid groups. As suggested by Tan and Lee [36], pretreatment at higher temperatures enhanced the solubility of cellulose and enzyme–cellulose interaction, thereby increasing the yields of glucose.

Our results are consistent with those reported by Peña et al. [35], who tested propyl-sulfonic acid as a solid catalyst to treat corn stover at significantly higher temperatures of 160, 180, and 200 °C, and observed glucose yields between 59% (160 °C) and 90% (180 °C). In our research, we also observed a glucose yield of up to 57.1% at a substantially lower pretreatment temperature of 90 °C when AC-pTSA was used. In a different study by Qian [28], sulfated zirconia (SA-J1) was employed (3 h at 150 °C) to pretreat rice straw, resulting in a maximum monosaccharide yield of 450 g kg^{-1}.

Combining these results with the characterization data, it appears that the presence of the multiple covalent bonds between C and O and S and O on the catalyst surface due to sulfonic acid treatment may have enhanced the hydrophilicity of the catalyst due to the formation of multiple active sites, as was also reported by Suganuma et al. [58]. Therefore, the resulting enhanced adsorptive interaction between the water-switchgrass slurry and the multiple surface-active sites (predominantly R-SO$_3$-H, R-COOH, and R-OH) may have altered the structure and porosity of switchgrass and possibly facilitated favorable enzymatic interaction to convert long-chain carbohydrates into simple sugars. Further, as also proposed by Tan and Lee [36], the surface groups may have removed

hydrogen linkages from cellulose, thereby lowering the crystallinity of biomass matrix resulting in enhanced enzymatic hydrolysis.

Figure 5. Glucose yields (%) after the enzymatic hydrolysis of switchgrass pretreated by sulfonic acid-supported carbon catalysts at various temperatures and reaction times.

3.3. Effect of Pretreatment Time on Glucose Production

The results also suggest that reaction time has a significant effect ($p < 0.05$) on glucose production. Overall, a longer reaction time allowed for better glucose yields. At the lowest temperature tested (30 °C), the increases in glucose yields observed after 90 to 120 min of pretreatment were between 0.06% and 10.77% for AC-SA, AC-MSA, and AC-

pTSA (Figure 5). Our results are similar to those of Qian [28], who also reported that the yield of monosaccharides increased by 12.5% when the reaction time was increased from 1 to 3 h when sulfated zirconia (SA-J1) was used as a catalyst at 150 °C. Tan and Lee [36] also reported an increased glucose yield from 97.6% to 99.8% with an increase in the pre-treatment time from 15 to 30 min (using Dowex Tr-G8), although a pretreatment time of 60 min resulted in a decrease in the yield of glucose (82.3%). Moreover, a longer reaction time between catalyst and switchgrass may have created additional porosity in the switchgrass matrix, which may have facilitated favorable adsorption by enzymes on switchgrass surface during hydrolysis. However, as the temperature was increased to 60 and 90 °C, the increase in glucose yield (when the time was increased from 90 to 120 min) was not as great (between 0.06% and 6.99%) as the increase that was observed at 30 °C, perhaps due to degradation of cellulose during longer pretreatment at higher temperatures, as was also observed by [28,36].

### 3.4. Effect of the Reusability of Catalyst on Glucose Production

Experimental data suggest that the catalysts were able to maintain activity even after they were reused three times. Overall, catalyst durability was found to be not significantly different between the catalysts' uses ($p = 0.18$). For the case of 90 °C, when AC-SA was employed as a catalyst, the change in conversion was not significant ($p > 0.05$) for 120 min (yield of 53.9% $\pm$ 1.01%), while for 90 minutes' pretreatment, change in conversion was significant ($p < 0.05$) corresponding to a yield of 49.9% $\pm$ 1.05%. Meanwhile, both AC-pTSA and AC-MSA exhibited similar results in which yields were not significant ($p > 0.05$) for a reaction time of 90 min, while data for 120 min were found to differ ($p < 0.05$) (Figure 5). The slight differences in catalytic activities observed for AC-pTSA and AC-MSA at 120 min may be attributed to repeated agitation of carbon particles that resulted in the breakdown of the structure and may have affected catalytic activity.

Our results are somewhat consistent with those reported by Tan and Lee [36], who reused Dowex (TM) Gr-8 six times for pretreating microalgae residue. The authors reported a glucose yield of about 94% after the fifth reuse, suggesting the unique benefits accorded by solid acid catalysts in biomass-to-energy processes.

3.5. Effect of Sulfonic Solid Acid Pretreatment on Delignification

Figure 6 presents plots for the delignification by all treatment conditions. The analysis of the data revealed that reaction time and temperature did not individually affect the delignification of switchgrass ($p = 0.1762$ and $p = 0.9735$) based on type 3 test of fixed effects. However, the interaction effect in combinations of catalyst, temperature, time, and reuse was significant ($p < 0.05$), suggesting that delignification varied with each temperature and reaction time. In addition, catalyst type and the number of times the catalyst was reused had a significant effect on the delignification of switchgrass ($p < 0.05$).

Despite exhibiting no clear trend, it was observed that activated carbon treated with AC-SA provided the least delignification (5.1–15.2%) when compared to AC- pTSA (14.0–24.8%) and AC-MSA (14.3–22.2%). However, it is interesting to note that despite minimal delignification, switchgrass pretreated with AC-SA provided glucose yields similar to AC-pTSA and AC-MSA. Similar results were observed by Yang et al. [14], who employed dilute sulfuric acid for the pretreatment of oven-dried switchgrass. The authors reported 39–68% glucose conversions despite low lignin removals (5.4–6.5%) that were attributed to simultaneous depolymerization and polymerization of lignin components when reacting with sulfuric acid. Besides, as proposed by Li et al. [59], it may be possible that sulfonated carbon catalysts may have disrupted the structure of lignin by the formation of hydrophobic and hydrophilic groups and may have subsequently enhanced the adsorptive interaction between cellulose and the enzyme. Therefore, from our results, it appears that sulfonic solid-impregnated carbon behaved somewhat similar to dilute acid for the pretreatment of switchgrass.
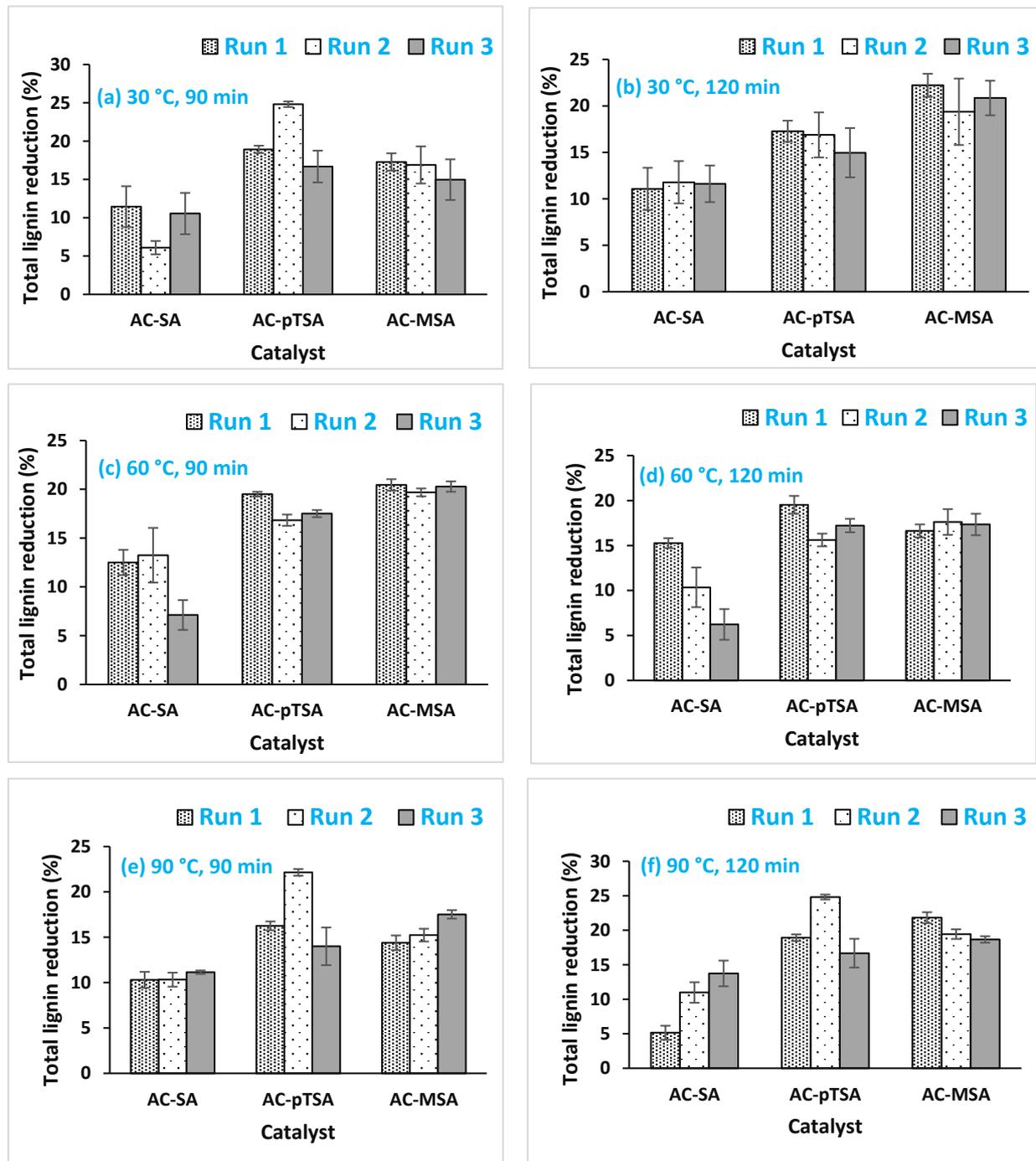
Figure 6. Total lignin reduction (%) after pretreatment of switchgrass via sulfonic acid-impregnated carbon catalysts at various temperatures and reaction times.

4. Conclusions

Switchgrass samples were pretreated with sulfonic acid-impregnated activated carbon catalysts for subsequent enzymatic conversion of glucan into glucose. Results indicated that sulfonic acid-impregnated activated carbon catalysts were effective for the pretreatment of switchgrass. At lower temperatures (30 and 60 °C), AC-SA was found to be more effective than AC-pTSA and AC-MSA, but no differences were found at 90 °C. However, the reaction time was found to significantly influence the pretreatment process. In addition, the catalysts were successfully reused three times with a minimal loss of activity. Our results suggest that sulfonic acid-impregnated activated carbon catalysts may potentially reduce

the use of acids for treatment and make biomass-to-alcohol operations more effective and environmentally friendly.

References

1. Kim, J.S.; Lee, Y.Y.; Kim, T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource Technol.* **2016**, *199*, 42–48. [CrossRef] [PubMed]
2. Shirkavand, E.; Baroutian, S.; Gapes, D.J.; Young, B.R. Combination of fungal and physicochemical processes for lignocellulosic biomass pretreatment—A review. *Renew. Sustain. Energ. Rev.* **2016**, *54*, 217–234. [CrossRef]
3. Su, T.; Zhao, D.; Khodadadi, M.; Len, C. Lignocellulosic biomass for bioethanol: Recent advances, technology trends, and barriers to industrial development. *Curr. Opin. Green Sustain. Chem.* **2020**, *24*, 56–60. [CrossRef]
4. Wright, L.; Turhollow, A. Switchgrass selection as a "model" bioenergy crop: A history of the process. *Biomass Bioenergy* **2010**, *34*, 851–868. [CrossRef]
5. Somerville, C.; Youngs, H.; Taylor, C.; Davis, S.; Long, S. Feedstocks for lignocellulosic biofuels. *Science* **2010**, *329*, 790–792. [CrossRef]
6. Wu, W.; Rondon, V.; Weeks, K.; Pullammanappallil, P.; Ingram, L.O.; Shanmugam, K.T. Phosphoric acid based pretreatment of switchgrass and fermentation of entire slurry to ethanol using a simplified process. *Bioresour. Technol.* **2018**, *251*, 171–180. [CrossRef] [PubMed]
7. Wang, F.; Shi, D.; Han, J.; Zhang, G.; Jiang, X.; Yang, M.; Wu, Z.; Fu, C.; Li, Z.; Xian, M.; et al. Comparative study on pretreatment processes for different utilization purposes of switchgrass. *ACS Omega* **2020**, *5*, 21999–22007. [CrossRef] [PubMed]
8. Jensen, K.; Clark, C.D.; Ellis, P.; English, B.; Menard, J.; Walsh, M.; Ugarte, D.L.T. Farmer willingness to grow switchgrass for energy production. *Biomass Bioenergy* **2007**, *31*, 773–781. [CrossRef]
9. Schmer, M.R.; Vogel, K.P.; Mitchell, R.B.; Perrin, R.K. Net energy of cellulosic ethanol from switchgrass. *Proc. Natl. Acad.Sci. USA* **2008**, *105*, 464–469. [CrossRef] [PubMed]
10. Gong, Z.; Yang, G.; Song, J.; Zheng, P.; Liu, J.; Zhu, W.; Huang, L.; Chen, L.; Luo, X.; Shuai, L. Understanding the promoting effect of non-catalytic protein on enzymatic hydrolysis effciency of lignocelluloses. *Bioresour. Bioprocess.* **2021**, *8*. [CrossRef]
11. Kothari, N.; Bhagia, S.; Pu, Y.; Yoo, C.G.; Mi, L.; Venketachalam, S.; Pattathil, S.; Kumar, R.; Cai, C.M.; Hahn, M.G.; et al. The effect of switchgrass plant cell wall properties on its deconstruction by thermochemical pretreatments coupled with fungal enzymatic hydrolysis or Clostridium thermocellum consolidated bioprocessing. *Green Chem.* **2020**, *22*, 7924. [CrossRef]
12. Karp, E.M.; Resch, M.G.; Donohoe, B.S.; Ciesielski, P.N.; O'Brien, M.H.; Nill, J.E.; Mittal, A.; Biddy, M.J.; Beckham, G.T. Alkaline pretreatment of switchgrass. *Sustain. Chem. Eng.* **2015**, *3*, 1479–1491. [CrossRef]
13. Kumar, S.; Kothari, U.; Kong, L.; Lee, Y.; Gupta, R. Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres. *Biomass Bioenergy* **2011**, *35*, 956–968. [CrossRef]
14. Yang, Y.; Sharma-Shivappa, R.; Burns, J.; Cheng, J. Dilute acid pretreatment of oven-dried switchgrass germplasms for bioethanol production. *Energy Fuels Energy Fuels* **2009**, 759–3766. [CrossRef]
15. Chen, H.; Liu, J.; Chang, X.; Chen, D.; Xue, Y.; Liu, P.; Lin, H.; Han, S. A review on the pretreatment of lignocellulose for high-value chemicals. *Fuel Process. Technol.* **2017**, *160*, 196–206. [CrossRef]
16. Kumar, P.; Barrett, D.; Delwiche, M.; Stroeve, P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* **2009**, 3713–3729. [CrossRef]
17. Huang, Y.; Fu, Y. Hydrolysis of cellulose to glucose by solid acid catalysts. *Green Chem.* **2013**, *15*, 1095–1111. [CrossRef]

18. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource. Technol.* **2005**, *96*, 673–686. [CrossRef]

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

20. Dien, B.S.; Jung, H.J.G.; Vogel, K.P.; Casler, M.D.; Lamb, J.F.S.; Iten, L.; Mitchell, R.B.; Sarath, G. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass Bioenergy* **2006**, *30*, 880–891. [CrossRef]

21. Wyman, C.E.; Balan, V.; Dale, B.E.; Elander, R.T.; Falls, M.; Hames, B.; Holtzapple, M.T.; Ladisch, M.R.; Lee, Y.Y.; Mosier, N.; et al. Comparative data on effects of leading pretreatments and enzyme loadings and formulations on sugar yields from different switchgrass sources. *Bioresour. Technol.* **2011**, *102*, 11052–11062. [CrossRef] [PubMed]

22. Chang, V.S.; Burr, B.; Holtzapple, M.T. Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* **1997**, *63*, 3–19. [CrossRef]

23. Alkaline Pretreatment of Coastal Bermudagrass for Bioethanol Production. Available online: https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1035&context=biosysengpres (accessed on 9 March 2021).

24. Alizadeh, H.; Teymouri, F.; Gilbert, T.I.; Dale, B.E. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). *Appl. Biochem. Biotechnol.* **2005**, *124*, 1133–1141. [CrossRef]

25. Kurakake, M.; Kisaka, W.; Ouchi, K.; Komaki, T. Pretreatment with ammonia water for enzymatic hydrolysis of corn husk, bagasse, and switchgrass. *Appl. Biochem. Biotechnol.* **2001**, *90*, 251–259. [CrossRef]

26. Vidal, P.F.; Molinier, J. Ozonolysis of lignin improvement of in vitro digestibility of poplar sawdust. *Biomass* **1988**, *16*, 1–17. [CrossRef]

27. Panneerselvam, A.; Sharma-Shivappa, R.R.; Kolar, P.; Ranney, T.; Peretti, S. Potential of ozonolysis as a pretreatment for energy grasses. *Bioresour. Technol.* **2013**, *148*, 242–248. [CrossRef] [PubMed]

28. Qian, E. Pretreatment and saccharification of lignocellulosic biomass. In *Research Approaches to Sustainable Biomass System*; Academic Press: Cambridge, MA, USA, 2013; pp. 181–204. ISBN 9780124046832.

29. Agbor, V.B.; Cicek, N.; Sparling, R.; Berlin, A.; Levin, D.B. Biomass pretreatment: Fundamentals toward application. *Biotechnol. Adv.* **2011**, *29*, 675–685. [CrossRef] [PubMed]

30. Guo, F.; Fang, Z. Solid- and Nano-Catalysts pretreatment and hydrolysis techniques. In *Pretreatment Techniques for Biofuels and Biorefineries*; Springer: Heidelberg, Germany; Dordrecht, The Netherlands; London, UK; New York, NY, USA, 2013; pp. 339–366. [CrossRef]

31. Ansanay, Y.; Kolar, P.; Sharma-Shivappa, R.R.; Cheng, J.J. Niobium oxide catalyst for delignification of switchgrass for fermentable sugar production. *Ind. Crops. Prod.* **2014**, *52*, 790–795. [CrossRef]

32. Zhou, C.; Xia, X.; Lin, C.; Tong, D.; Beltramini, J. Catalytic conversion of lignocellulosic biomass to fine chemicals and fuels. *Chem. Soc. Rev.* **2011**, *40*, 5588–5617. [CrossRef]

33. Guo, F.; Fang, Z.; Xu, C.; Smith, R. Solid acid mediated hydrolysis of biomass for producing biofuels. *Prog. Energy Combust. Sci.* **2012**, *38*, 672–690. [CrossRef]

34. Qi, B.; Vu, A.; Wickramasinghe, S.R.; Qian, X. Glucose production from lignocellulosic biomass using a membrane-based polymeric solid acid catalyst. *Biomass Bioenergy* **2018**, *117*, 137–145. [CrossRef]

35. Peña, L.; Xu, F.; Hohn, K.; Li, J.; Wang, D. Propyl-sulfonic acid functionalized nanoparticles as catalyst for pretreatment of corn stover. *J. Biomater. Nanobiotechnol.* **2014**, *5*, 8–16. [CrossRef]

36. Tan, I.S.; Lee, K.T. Solid acid catalysts pretreatment and enzymatic hydrolysis of macroalgae cellulosic residue for the production of bioethanol. *Carbohyd Polym.* **2015**, *124*, 311–321. [CrossRef] [PubMed]

37. Qi, W.; He, C.; Wang, Q.; Liu, S.; Yu, Q.; Wang, W.; Leksawasdi, N.; Wang, C.; Yuan, Z. Carbon-based solid acid pretreatment in corncob saccharification: Specific xylose production and efficient enzymatic hydrolysis. *ACS Sustain. Chem. Eng.* **2018**, *6*, 3640–3648. [CrossRef]

38. Hara, M. Biomass conversion by a solid acid catalyst. *Energy Environ. Sci.* **2010**, *3*, 601–607. [CrossRef]

39. Li, Z.; Su, K.; Ren, J.; Yang, D.; Cheng, B.; Kim, C.K.; Yao, X. Direct catalytic conversion of glucose and cellulose. *Green Chem.* **2018**, *20*, 863–872. [CrossRef]

40. Huang, F.; Li, W.; Zhang, T.; Li, D.; Liu, Q.; Zhu, X.; Ma, L. Conversion of biomass-derived carbohydrates into 5-hydroxymethylfurfural catalyzed by sulfonic acid-functionalized carbon material with high strong-acid density in γ-valerolactone. *Res. Chem. Intermed.* **2018**, *44*, 5439–5453. [CrossRef]

41. Alam, M.I.; De, S.; Khan, T.S.; Haider, M.A.; Saha, B. Acid functionalized ionic liquid catalyzed transformation of non-food biomass into platform chemical and fuel additive. *Ind. Crops. Prod.* **2018**, *123*, 629–637. [CrossRef]

42. Wu, Y.; Fu, Z.; Yin, D.; Xu, Q.; Liu, F.; Lu, C.; Mao, L. Microwave-assisted hydrolysis of crystalline cellulose catalyzed by biomass char sulfonic acids. *Green Chem.* **2010**, *12*, 696–700. [CrossRef]

43. Wang, J.; Xu, W.; Ren, J.; Liu, X.; Lu, G.; Wang, Y. Efficient catalytic conversion of fructose into hydroxymethylfurfural by a novel carbon-based solid acid. *Green Chem.* **2011**, *13*, 2678. [CrossRef]

44. Rackemann, D.W.; Bartley, J.P.; Doherty, W.O.S. Methanesulfonic acid-catalyzed conversion of glucose and xylose mixtures to levulinic acid and furfural. *Ind. Crop. Prod.* **2014**, *52*, 46–57. [CrossRef]

45. Rackemann, D.W.; Bartley, J.P.; Harrison, M.D.; Doherty, W.O.S. The effect of pretreatment on methanesulfonic acid-catalyzed hydrolysis of bagasse to levulinic acid, formic acid, and furfural. *RSC Adv.* **2016**, *6*, 74525. [CrossRef]

46. Zhang, B.; Ren, J.; Liu, X.; Guo, Y.; Guo, Y.; Lu, G.; Wang, Y. Novel sulfonated carbonaceous materials from p-toluenesulfonic acid/glucose as a high-performance solid-acid catalyst. *Catal. Commun.* **2010**, *11*, 629–632. [CrossRef]

47. Evangelin, C.; Naren, S.; Dharmendirakumar, M. Comparison of the Surface features of the three chemically modified silk cotton hull activated carbons. *Orient. J. Chem.* **2012**, *28*, 1761–1768, ISSN 0970-020 X. [CrossRef]

48. Zhang, W.; Tao, H.; Zhang, B.; Ren, J.; Lu, G.; Wang, Y. One-pot synthesis of carbonaceous monolith with surface sulfonic groups and its carbonization/activation. *Carbon* **2011**, *49*, 1811–1820. [CrossRef]

49. Reye, J.T.; Lu, J.; Maxwell, K.E.; Banerjee, S. Enhancement of cellulase catalysis of wood pulp fiber by cationic polyelectrolytes. *Biomass Bioenergy* **2011**, *35*, 4887–4891. [CrossRef]

50. Sluiter, A.; Hames, B.D.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of structural carbohydrates and lignin in biomass. In *Laboratory Analytical Procedure (LAP)*; National Renewable Energy Laboratory: Golden, CO, USA, 2008.

51. Liu, X.; Huang, M.; Ma, H.; Zhang, Z.; Gao, J.; Zhu, Y.; Han, X.; Guo, X. Preparation of a carbon-based solid acid catalyst by sulfonating activated carbon in a chemical reduction process. *Molecules* **2010**, 7188–7196. [CrossRef] [PubMed]

52. Shu, Q.; Zhang, Q.; Xu, G.; Nawaz, Z.; Wang, D.; Wang, J. Synthesis of biodiesel from cottonseed oil and methanol using a carbon-based solid acid catalyst. *Fuel Process Technol.* **2009**, *90*, 1002–1008. [CrossRef]

53. Liang, X.; Zeng, M.; Qi, C. One-step synthesis of carbon functionalized with sulfonic acid groups using hydrothermal carbonization. *Carbon* **2010**, *48*, 1844–1848. [CrossRef]

54. Kitano, M.; Yamaguchi, D.; Suganuma, S.; Nakajima, K.; Kato, H.; Hayashi, S.; Hara, M. Adsorption-enhanced hydrolysis of β-1,4-glucan on graphene-based amorphous carbon bearing SO_3H, COOH, and OH Groups. *Langmuir* **2009**, *25*, 5068–5075. [CrossRef]

55. Nasef, M.M.; Saidi, H. Surface studies of radiation grafted sulfonic acid membranes: XPS and SEM analysis. *Appl. Surf. Sci.* **2006**, *252*, 3073–3084. [CrossRef]

56. Mo, X.; Lotero, E.; Lu, C.; Liu, Y.; Goodwin, J.G. A novel sulfonated carbon composite solid acid catalyst for biodiesel synthesis. *Catal Lett.* **2008**, *123*, 1–6. [CrossRef]

57. Alvear-Daza, J.J.; Pasquale, G.A.; Rengifo-Herrera, J.A.; Romanelli, G.P.; Pizzio, L.R. Mesoporous activated carbon from sunflower shells modified with sulfonic acid groups as solid acid catalyst for itaconic acid esterification. *Catal. Today* **2021**, in press. [CrossRef]

58. Suganuma, S.; Nakajima, K.; Kitano, M.; Yamaguchi, D.; Kato, H.; Hayashi, S.; Hara, M. Hydrolysis of cellulose by amorphous carbon bearing SO3H, COOH, and OH groups. *J. Am. Chem. Soc.* **2008**, *130*, 12787–12793. [CrossRef]

59. Li, X.; Luo, X.; Li, K.; Zhu, J.; Fougere, J.; Clarke, K. Effects of SPORL and dilute acid pretreatment on substrate morphology, cell physical and chemical wall structures, and subsequent enzymatic hydrolysis of lodgepole. *Appl. Biochem. Biotechnol.* **2012**, *168*, 1556–1567. [CrossRef]