Metal Oxides and Ultraviolet Light-based Photocatalytic Pretreatment of Biomass for Biogas Production and Lignin Oxidation

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Lignocellulosics are abundant and readily available as the raw material for the production of biogas. However, the structure of this raw material needs to be modified to increase its digestibility during anaerobic fermentation. Various pretreatment methods that have been proposed in the past have been examined; however, the focus of the present study was to pretreat a wheat straw (WS) substrate using an advanced oxidation process (AOP) with a metal oxide photocatalyst combined with ultraviolet (UV) irradiation. Four different metal oxides were examined at 0, 1, 2, 3, and 4% dosages (w/w) coupled with UV irradiation for 0, 60, 120, and 180 min. Experimental results revealed that among all metal oxide catalysts examined, only the 4% CuO combined with 180 min UV irradiation caused the most lignin to be released from the WS. This resulted in the highest vanillic acid (VA) being produced (4.32 ± 0.15 mg VA/g VS). This WS pretreatment also resulted in a biomethane potential (BMP) assay of 384 ± 16 NmL CH_4/g VS. The BMP assay results revealed a maximum 28% increase in biodegradability and a 57% increase in methane production. The use of either metal oxide catalysts or UV irradiation alone resulted in ineffective WS pretreatment.

Keywords: Photocatalysis; Wheat straw; Metal oxides; Lignin oxidation; UV light

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INTRODUCTION

There is much discussion on the use of renewable energy resources to replace fossil fuels that are more environmentally sustainable and carbon neutral. For these reasons, lignocellulosic biomasses represent a better raw material to produce biofuels to possibly replace non-renewable fossil fuels. However, there is a hindrance accompanying the use of this rich biomass, which is the close structural packing fibers of cellulose, hemicellulose, and lignin. The primary bottleneck involves the breakdown of the recalcitrant matrix of lignocellulosic constituents prior to biodegradation treatments (Akhtar *et al*. 2016). Different pretreatments have been developed that include chemical, biological, and physical methods. The main purpose of these methods is to alter and/or remove lignin and hemicelluloses, to decrease cellulose crystallinity, and to maximize enzymatic action by increasing the surface area of the substrate (Kumari and Singh 2018). Among the above-mentioned pretreatments, most are accompanied by different barriers. Some of these include the need for high temperature and increased pressure, or the utilization of various compounds that yield noxious substances in the process of fermentation (Clark and Mackie 2007).

The oxidation of lignocellulosic biomass in the presence of a catalyst can represent a very favorable pretreatment method. These mild oxidation conditions can involve the use of various reaction pressures, temperatures, and pH values without introducing harmful substances into the oxidized sample. Several studies have focused on the oxidation of lignin and its subsequent conversion to valuable products (Sainsbury *et al.* 2013). Hence, a complex lignin polymer is converted to low molecular weight fragments that contain phenolic groups.

For lignin photocatalysis under mild conditions, advanced oxidation processes (AOP) can also be used as an alternate solution, in which photocatalysis driven redox reactions are used to decompose and subsequently oxidize lignin to products of economic interest (*e.g.*, vanillic acid, *p*-coumaric acid, benzoic acid, vanillin, *etc*.) with vanillic acid as major product of oxidation (Ksibi 2003). A considerable amount of work has been done to produce low-cost, stable, practically feasible and efficient photocatalysts (Rawalekar and Mokari 2013). One of the most studied photocatalysts is titanium dioxide (TiO_2), which absorbs light in the form of rutile or anatase in the ultraviolet (UV) region, but still its photo-catalysis, stability, and band gap is variable if its composites are made with different metal oxides (Kazuhito *et al.* 2005). However, the light scattered by the rutile TiO_2 is more than it absorbs; thus it is rarely used as a photocatalyst. This form of TiO_2 is mainly used in paints and pigments. Anatase TiO_2 is considered to be very active because of its surface chemistry and its higher conduction band energy (Hoffmann *et al.* 1995).

Titanium dioxide is generally considered as a highly effective catalyst for detoxifying organic contaminants contained in wastewater, when activated by ultraviolet (UV) light (Al-Dawery 2013). With emerging catalysis technologies, more potential candidates are introduced that can disrupt biomass bonding and increase the digestibility of lignocellulosic materials. In addition, the excitation of a photocatalyst with electromagnetic energy that is higher than its bandwidth is considered essential to the photo-oxidation process. Furthermore, many advantages have been reported with titanium dioxide (TiO_2), copper oxide (CuO), and zinc oxide (ZnO) photocatalysts; these advantages include low toxicity, improved solubility, low cost, high stability, ease of excitation, and ability to make thin layers on steel or glass (Kumar and Devi 2011). In the photo-oxidation process, water molecules are fragmented into radicals due to the photon energy absorbed by photocatalysts that form electron-hole pairs. The hydroxyl radical ($HO^\bullet$) formed in this process can completely demineralize various organic contaminants (*e.g.*, organic acids, amines, and dyes) (Lacombe and Keller 2012).

Titanium dioxide and other metal oxides, such as CuO, iron (III) oxide (Fe_2O_3), and ZnO, have been reported to act as photocatalysts. Titanium dioxide use in paints and dyes is well known, but it is also used in cosmetics and antimicrobial products due to its photocatalytic activity (Nations *et al.* 2011). The design of the present study is to explore the photocatalytic properties of various commercially available photocatalysts to disturb the complex structure of wheat straw (WS) and promote biogas production with improved feed stock digestibility. This study was also designed to complete further work based on an earlier investigation (Alvarado-Morales *et al.* 2017). Moreover, the current study aimed to identify a potentially more favorable catalyst for the photo-oxidation of lignin contained in the biomass substrate.

EXPERIMENTAL

Materials

Characteristics of inoculum and WS

Inoculum was taken from a local biogas plant situated at the PMAS-Arid Agriculture University, Rawalpindi Pakistan, and was incubated at thermophilic conditions and degassed prior to experimental use. The inoculum was also subjected to various tests that resulted in total volatile fatty acids (TVFAs) 8.23, 0.3 ± 0.0 g/L, total solids (TS) 26.3 ± 2.1 g/L, and volatile solids (VS) 16.6 ± 1.4 g/L. Among the VFAs, acetate was 0.15 ± 0.0 g/L, whereas all other VFAs were at negligible concentration levels. Moreover, the ammoniacal nitrogen (NH_4-N) and total Kjeldahl nitrogen were measured as 3.1 ± 0.2 g/L and 2.9 ± 0.1 g/L, respectively.

The WS was collected from a local farm in the district of Sahiwal (Pakistan). The straw was dried, cut, and sieved into 3- to 4-cm segments using a CM1000 Cutting Mill (Laarmann Group, B.V., Roermond, Netherlands). The cut WS was stored in plastic bags at 4 °C until needed. The WS was also analyzed for its basic properties. The measured results were 93.1% ± 1.2% TS and 87.4% ± 1.6% VS (fresh samples). The Klason lignin was measured as 26.7% ± 0.3% of TS, whereas cellulose and hemicelluloses were 43.3% ± 0.7% of TS and 29.6% ± 0.6% of TS, respectively.

Advanced oxidation process (AOP) pretreatments

The pretreatments were performed in accordance to the previous published method of Alvarado-Morales *et al.* (2017). Specifically, the samples were prepared by soaking 0.93 g of WS in 240 mL of water while continuously stirring at 200 rpm to evenly distribute the solids. The slurry was placed 30 cm away from a UV-lamp (SUV-100P; UV Superstore, Inc., Winder, GA, USA)

The UV beams were collimated using a hollow tube (Fig. 1) to increase the efficacy of the pretreatments (Hansen *et al.* 2013). All the pretreatments were subject to an initial temperature of 25 °C. Four different photocatalysts were obtained from Sigma Aldrich (Darmstadt, Germany). The four catalysts, TiO_2, CuO, Fe_2O_3, and ZnO were used to pretreat the WS at concentration dosages of 0, 1, 2, 3, or 4% (w/w on dry WS). The WS with photocatalyst was exposed to UV light for 0, 60, 120, or 180 min for pretreatment. After the pretreatments, three fourths of the pretreated sample was subject to biomethane potential (BMP) experiments to evaluate methane production from the pretreated samples; the experiments were repeated three times. While the remaining one fourth of the pretreated sample was subject to further analysis *via* SEM and vanillic acid (VA) analysis to justify the pretreatment.

BMP assay

Biomethane potential assays were conducted on pretreated samples and controls using the protocol described by Angelidaki *et al.* (2009) at mesophilic conditions. The 327-mL ± 1 mL glass bottles, with 100 mL of working volume, were prepared for BMP assays, with 40% of inoculum and a 2 g VS/L organic loading rate (OLR).

Bacterial colonies usually form dense aggregations and are not evenly distributed if the bioreactors are not agitated. Hence, the bioreactors were manually shaken twice a day. Each reactor's accuracy was validated using a positive control with Avicel® PH-101 cellulose (Sigma Aldrich, Darmstadt, Germany). Glass bottles with 80 mL of inoculum and 120 mL of water were used to determine the residual methane of all samples. Furthermore, the glass bottles were flushed with nitrogen gas and sealed with rubber corks. Every BMP assay was repeated in triplicate to ensure the reproducibility of the measured results.

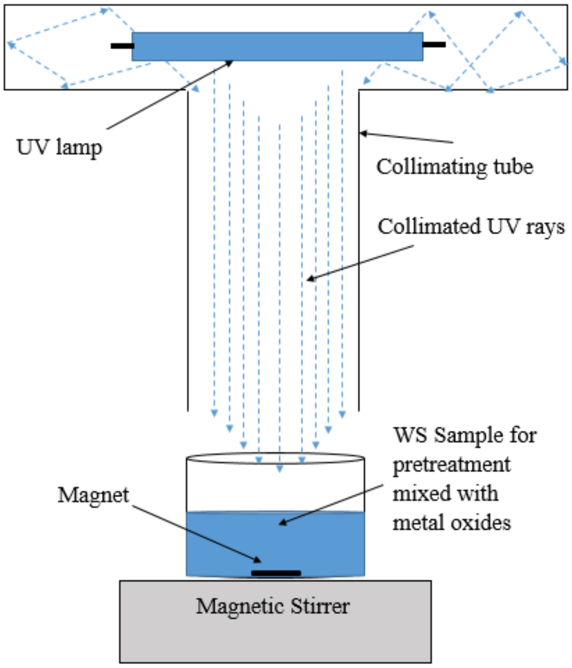


Fig. 1. Schematic of the pretreatment set-up used for UV irradiation of the WS samples with photocatalyst

Methods

The determinations of NH_4-N, total Kjeldahl nitrogen, pH, VS, and TS were conducted according to standard protocols as described by the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2011). A Fisher FE150 pH meter (Fisher Scientific, Hampton, NH, USA) was used to measure the pH of the samples. The VFAs analysis of the inoculum was performed using the protocol specified by Kougias *et al.* (2014) using an internal standard (4-methylvaleric acid). The procedure used a gas chromatograph (GC) with an auto-sampler (Kugelman and McCarty 1965). A Clarus 500 GC instrument (Perkin-Elmer, Waltham, MA, USA) was operated with a split/split-less injector. The methane contents from the BMP reactors were investigated using a (flame ionization detector (FID, Agilent Technologies, Waldbronn, Germany). The separation was completed using a glass capillary column with the dimensions of 3 m × 6 mm and with an inner diameter of 2.5 mm (Restek, Bellefonte, PA, USA). The GC temperatures were 110 °C and 160 °C for the injection and the detection ports, respectively. Klason lignin and carbohydrates were determined using NREL standard protocols (Sluiter *et al.* 2011).

Strong acid hydrolysis was followed by elemental analysis using a vario MACRO cube elemental analyzer (Elementar, Langenselbold, Hesse, Germany) to determine macro- and micro-nutrients (*e.g.*, trace metals) in the wheat straw (Awais *et al.* 2016). The determinations of elemental levels of carbon, hydrogen, nitrogen, oxygen, and sulphur, as well as C/N ratio, were performed using a vario MACRO cube elemental analyzer (Elementar, Langenselbold, Hesse, Germany). All determinations were performed in triplicate to determine experimental error/variability. An UltiMate 3000 ultra-high performance liquid chromatograph (UHPLC; Thermo Fisher Scientific, Waltham, MA, USA) coupled with a Dionex UltiMate 3000 multiple wavelength detector (Thermo Fisher Scientific) was used to evaluate the products that arose from lignin oxidation. Separations with the UHPLC were completed using a BDS Hypersil C18 reverse phase column (4.6 mm × 100 mm) and 5 µm particle size (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a BDS Hypersil C18 guard column (4 mm × 10 mm) and 5 µm particle size (Thermo Fisher Scientific, Waltham, MA, USA). At a constant flow rate of 1 mL/min in 0.3% (v/v) acetic acid gradient, the separation was achieved. During the separation of contents, the compartment temperature was set at 30 °C, and injection volume was 20 µL. The surface analysis of the nanocomposites and wheat straw after pretreatment were performed using a thermionic tungsten gun equipped with a scanning electron microscope (SEM-FEI Inspect S; FEI Co., Hillsboro, OR, USA) fitted with large field detectors operating under high vacuum mode.

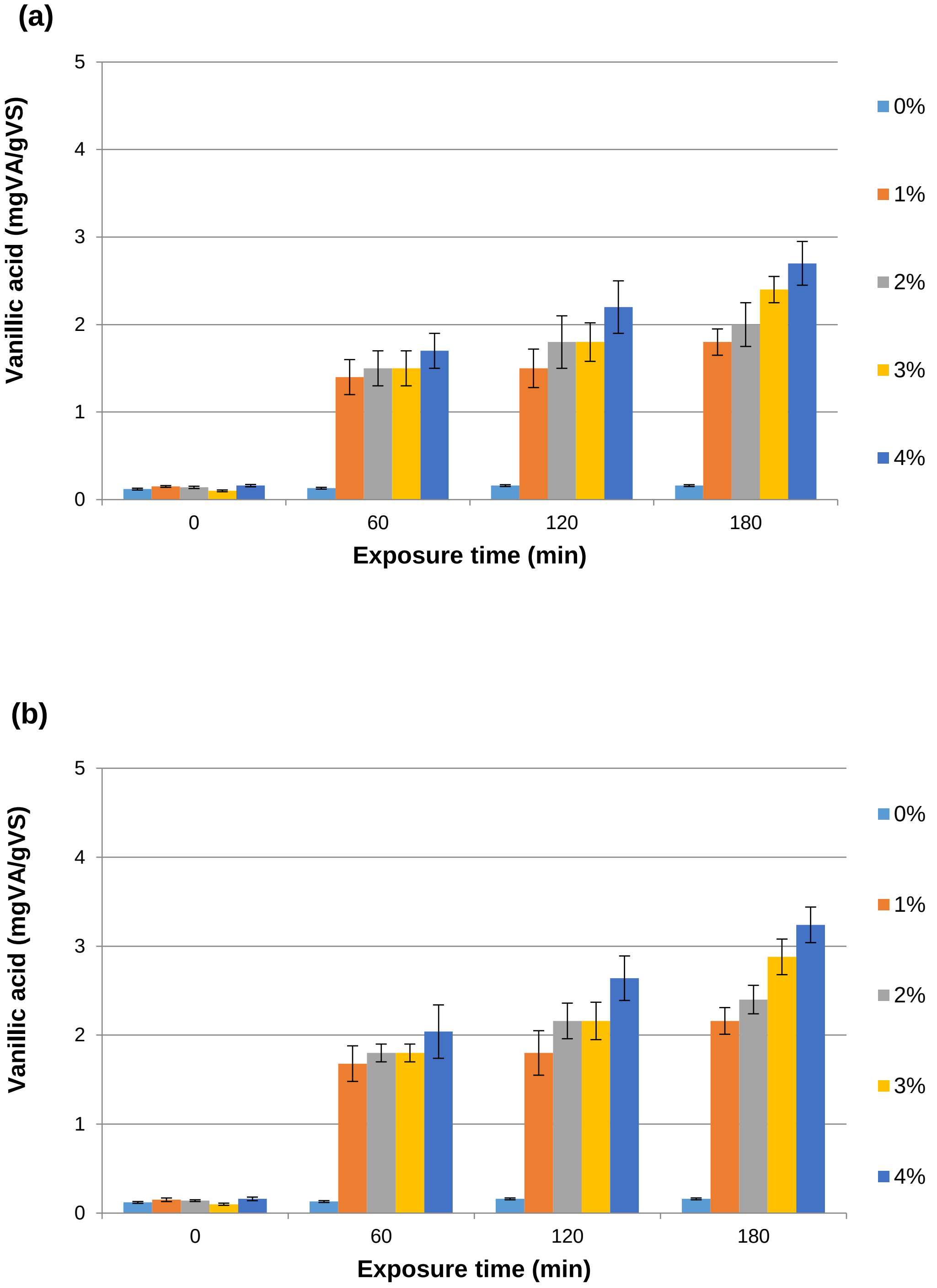
Statistical analysis

Descriptive statistical measures, means, and standard deviations of all the raw data sets (treated and untreated samples) were analyzed using Microsoft Excel software (2007 version, Microsoft Corp., Redmond, WA, USA). A one-way analysis of variance (ANOVA) was used to compare the means of the data sets using a $p < 0.05$ significance level.

RESULTS AND DISCUSSION

Lignin Oxidation

Lignin oxidation was performed to justify the ability of metal oxides and UV light to cause pretreatment of wheat straw. The control treatments resulted in non-significant ($p < 0.05$) amounts of produced VA. With a consistent increase in monocatalyst TiO_2 concentration, a non-significant increase in lignin oxidation was also observed. The control treatment containing 1, 2, 3, and 4% TiO_2 was not exposed to the UV light; therefore no significant change was observed in VA concentration (0.12, 0.15, 0.14, 0.1, and 0.16 mg VA/g VS). Another set of experiments was designed to observe the VA production from lignin oxidization under UV irradiation (60 min) using various concentrations of TiO_2 catalyst (0 to 4%). It was concluded that with increasing catalyst concentration and UV exposure, pretreatment extent was increased. When the WS was pretreated with 120 min of UV irradiation with 4% TiO_2 catalyst, the VA production was 2.2 ± 0.1 mg VA/g VS, whereas with 0% TiO_2 the VA production was only 0.16 ± 0.0 mg VA/g VS. The maximum VA production of 2.7 ± 0.1 mg VA/g VS was observed when 4% TiO_2 and 180 min of UV irradiation were used. Earlier results revealed that increasing the UV light exposure time increased lignin oxidation, which was presumably caused by the generation of more hydroxyl radicals (Portjanskaja *et al.* 2006). The observed increased VA production corresponded to morphological changes observed with the solid surfaces that were caused by 4% TiO_2 and 180 min of UV irradiation pretreatment (*versus* the untreated control) (Fig. 3). A second set of experiments involved pretreating the WS with ZnO catalyst at various conditions as described earlier. The control treatment with no UV exposure resulted in insignificant amounts of VA production ($p < 0.05$). Oxidation of lignin increased as the UV exposure time increased when the ZnO catalyst was used. When 0% ZnO catalyst was used, the VA yields were insignificant ($p < 0.05$).


(a)
Vanillic acid (mgVA/gVS)
5
4
3
2
1
0
0
60
120
180
Exposure time (min)
0%
1%
2%
3%
4%
(b)
Vanillic acid (mgVA/gVS)
5
4
3
2
1
0
0
60
120
180
Exposure time (min)
0%
1%
2%
3%
4%

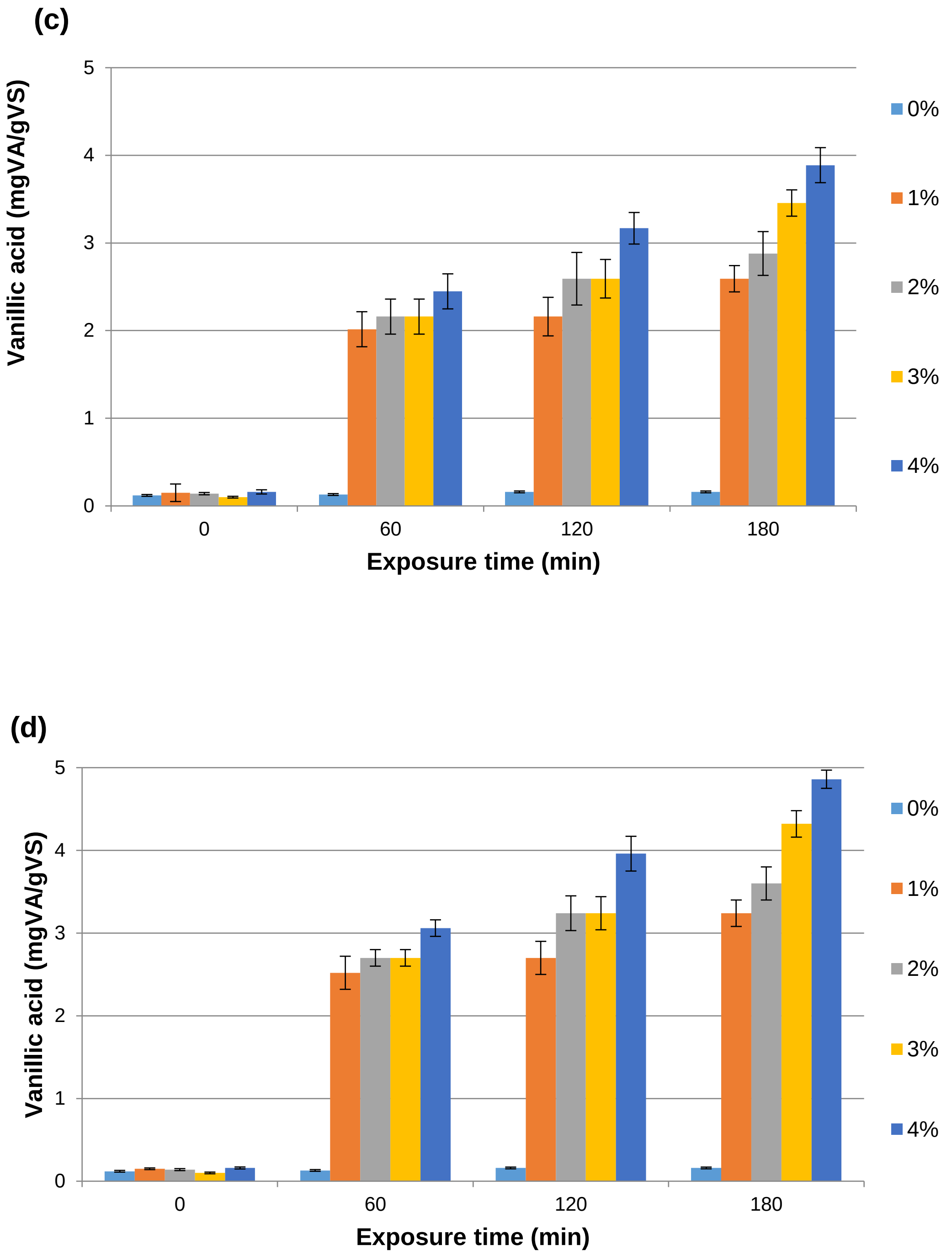
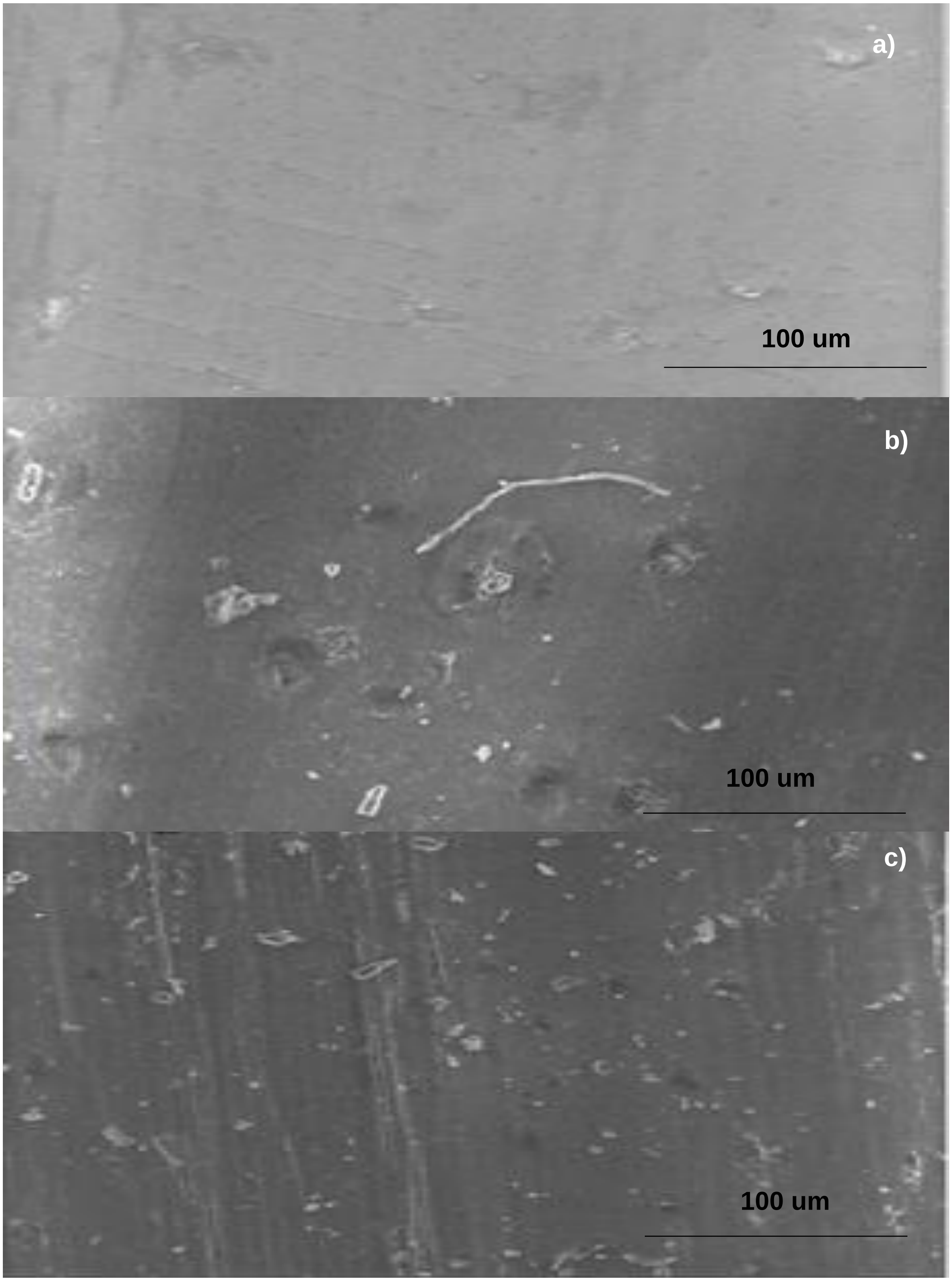


Fig. 2. Comparisons of different pretreatment conditions using various photocatalysts on VA production from lignin oxidation based on the total VS (a) TiO_2 Pretreatment (b) ZnO Pretreatment (c) Fe_2O_3 Pretreatment (d) CuO Pretreatment


a)
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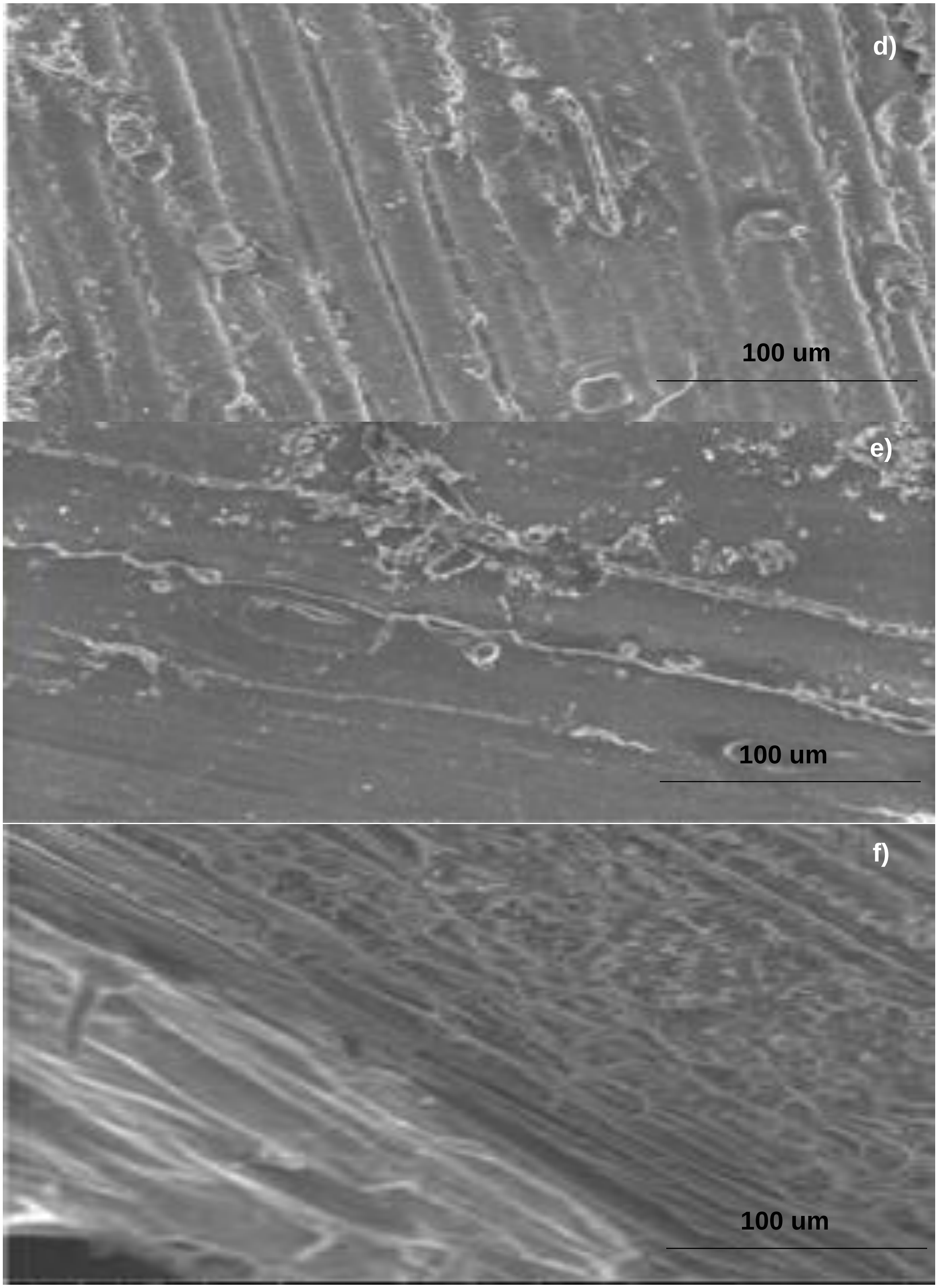


Fig. 3. SEM images of WS: (a) double negative control; (b) only 180 min UV irradiation (no catalyst); (c) pretreated with 4% TiO_2 and 180 min UV irradiation; (d) pretreated with 4% ZnO and 180 min UV irradiation; (e) pretreated with 4% Fe_2O_3 and 180 min UV irradiation; and (f) pretreated with 4% CuO and 180 min UV irradiation

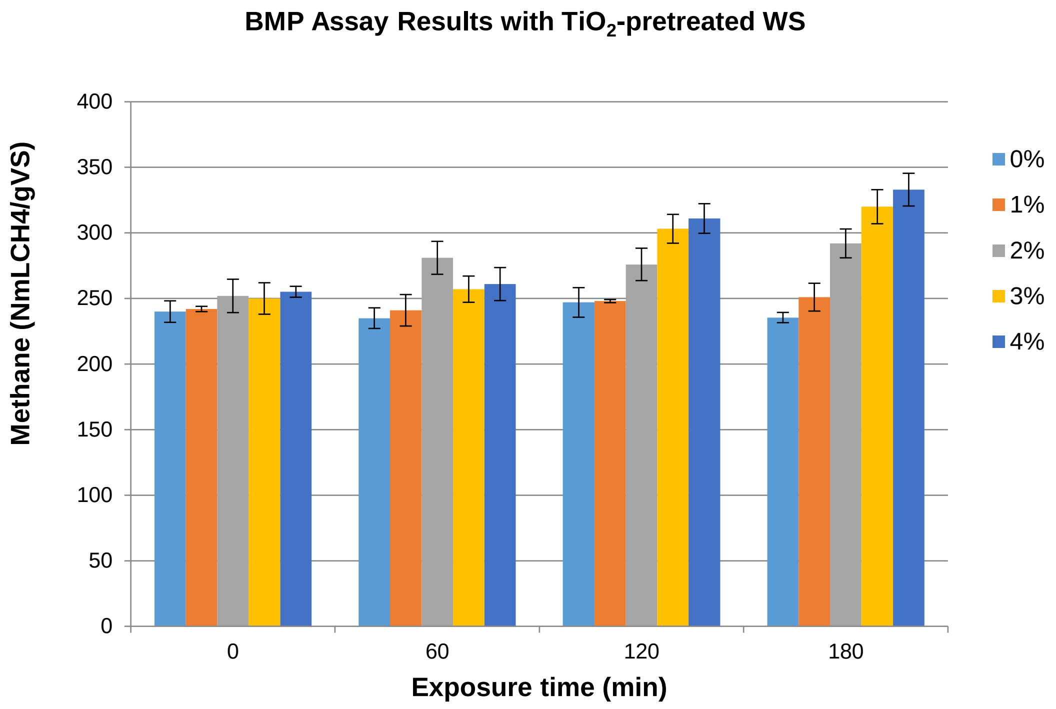
Significantly higher concentrations of VA (3.24 ± 01 mg VA/g VS) were detected when 4% ZnO catalyst and 180 min of UV irradiation time was used. This trend was similar to the experiments conducted with the TiO_2 catalyst, but the ZnO catalyst had higher VA production *versus* the TiO_2 catalyst. The SEM analysis of the ZnO-pretreated sample also offered more lignin release from biomass compared to TiO_2 alone, so the escape of lignin from biomass enhanced the surface area for enzymatic degradation.

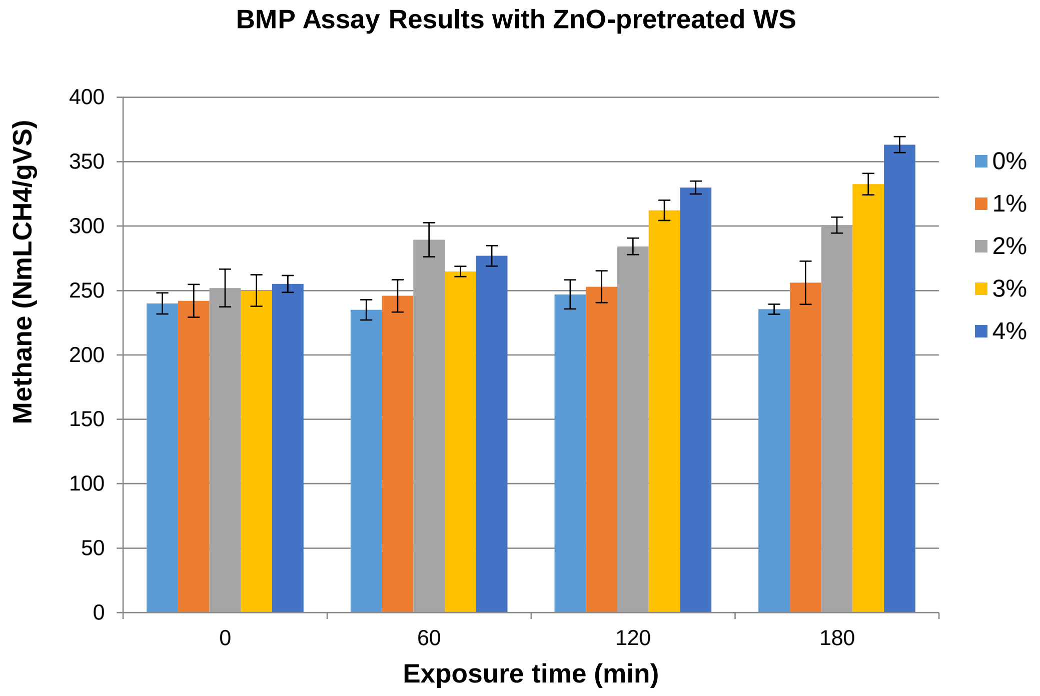
The Fe_2O_3 catalyst was used at various concentrations and UV exposure times to check the catalyst's potential to modify the WS surfaces. In the negative controls, the VA production was insignificant and revealed the legitimacy of the pretreatments as the UV and catalyst separately posed no significant effect on VA production. The trends showed increased VA production with increased UV exposure times and Fe_2O_3 catalyst concentration. More specifically, the 4% Fe_2O_3 catalyst yielded a maximum 3.89 ± 0.12 mg VA/g VS after 180 min of UV exposure (Fig. 2c). These results were also observed in the SEM images of the modified WS surfaces when Fe_2O_3 was used in place of either ZnO or TiO_2. The more extensive modifications to surfaces when Fe_2O_3 catalyst is used are presumed to cause more lignin to escape from the WS (Fig. 3) than with ZnO or TiO_2; thus, it allowed for further lignin oxidation (*i.e.*, VA production).

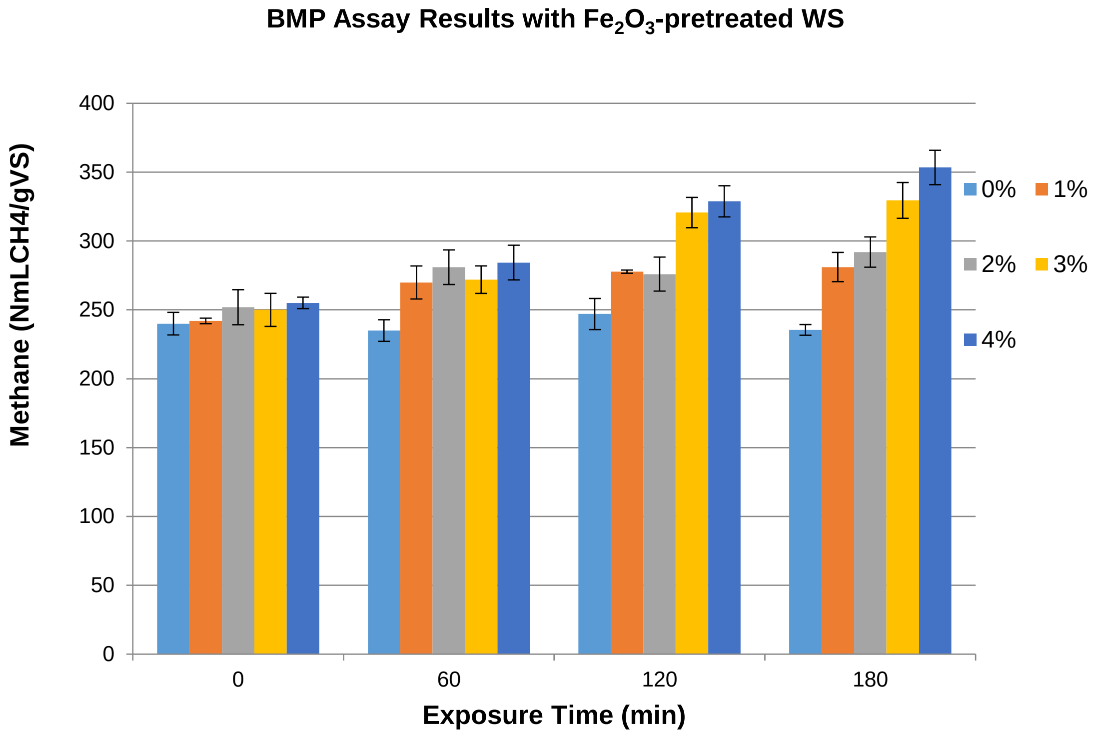
To speed up the reportedly slow and incomplete process of lignin oxidation, as reported by Deublein and Steinhauser (2011), CuO was examined in this study in conjunction with UV exposure to develop a more potent AOP pretreatment for WS. Using CuO catalyst with UV radiation resulted in a more extensive pretreatment of the WS when compared to all other UV photocatalysts examined in this study. The SEM images revealed that lignin fragments could diffuse more readily when CuO was used as the catalyst. The comparisons of the SEM images of Fig. 3 illustrated that CuO extensively modified the WS surfaces and loosened more of the bound fibers *versus* the other metal oxide catalysts at same conditions. This trend also highly affected the biodegradability of wheat straw and a treatment of 180 min of UV exposure with 4% CuO resulted in 4.32 ± 0.15 mg VA/g VS (Fig. 2d). This VA production confirmed that 4% CuO and UV exposure for 180 min resulted in the highest pretreatment and lignin oxidation afterwards, among all the pretreatments. The general trends indicated that lignin oxidization increased when the CuO catalyst concentration increased and when the UV irradiation time increased. Moreover, separately, CuO and UV light were not associated with any lignin oxidation, as the results of separate analysis showed insignificant differences (Fig. 2d).

BMP Assay Results with WS Pretreated with Various AOPs

The WS from the various AOP pretreatments was subjected to anaerobic digestion to produce biomethane. The pretreatment's effect of and validation of lignin oxidation were also determined by subjecting all the pretreatments to biomethane potential assays. The only TiO_2-pretreated WS was also subjected to BMP assays. A control treatment (*i.e.*, no photocatalyst and 0 min UV irradiation) was conducted with the WS. The biogas production from the WS control was 240 NmL CH_4/g VS (standardized/normalized volumetric flow rate). The TiO_2 catalyst was increased in $\Delta 1\%$ increments, and the pretreatments were completed without UV irradiation (0 min). The amount of methane production was insignificant when compared to the control treatment ($p < 0.05$) (Fig 4a). Generally, it can be observed that TiO_2 catalyst alone does not significantly augment methane production (Alvarado-Morales *et al.* 2017). Using the combination of 4% TiO_2 and 180 min of UV irradiation yielded 2.7 ± 0.1 mg VA/g VS and 333 ± 15 NmL CH_4/g VS (*i.e.*, 33% increase over control).


BMP Assay Results with TiO_2-pretreated WS
Methane (NmLCH4/gVS)
400
350
300
250
200
150
100
50
0
0
60
120
180
Exposure time (min)
0%
1%
2%
3%
4%


BMP Assay Results with ZnO-pretreated WS
Methane (NmLCH4/gVS)
400
350
300
250
200
150
100
50
0
0
60
120
180
Exposure time (min)
0%
1%
2%
3%
4%



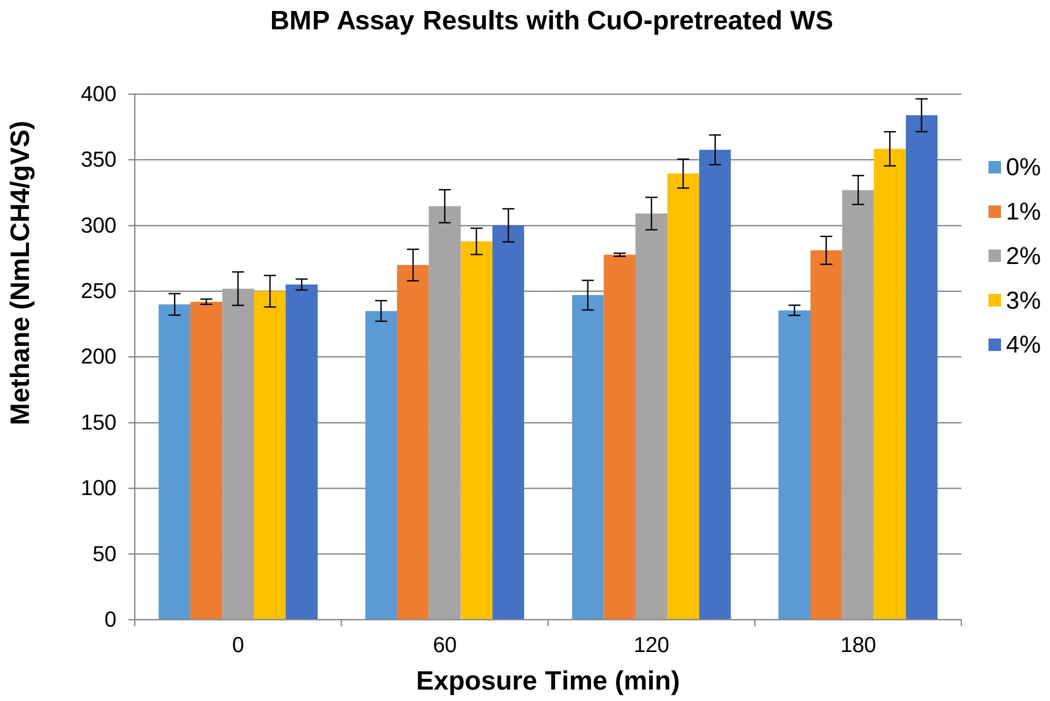


Fig. 4. Methane production during BMP assays with WS substrate treated with various pretreatment conditions and photocatalysts

The pretreated WS with 4% TiO_2 and 180 min of UV irradiation was observed to have more pits and furrows on its surface *versus* the untreated WS sample. Moreover, Carrere *et al.* (2016) indicated that the goals of biomass pretreatment are to remove lignin, reduce cellulose crystallinity, and increase substrate surface area for enzymes to interact.

These observations established that increasing lignin escape and the development of pits on the WS surface increased the digestibility of WS under BMP assay.

In this section, ZnO was applied to increase the efficiency of lignin oxidation. No appreciable changes were observed in the control treatments with ZnO when compared to control treatments with TiO_2. Methane production increased when the UV irradiation time increased for the various catalyst dosage levels. The production increased from 246 ± 12 NmL CH_4/g VS using 60 min UV irradiation and 1% ZnO to a maximum of 353 ± 17 NmL CH_4/g VS (*i.e.*, 41% increase) using 4% ZnO for 180 min UV irradiation (Fig. 4b). The SEM analysis of the maximum pretreated sample revealed that the development of pits and escape of lignin were greater compared to the untreated and TiO_2-pretreated WS under the same conditions. This can be explained by the fact that a photocatalyst offering more surface disruption alongside a pretreatment usually produces more hydroxyl radicals ($HO^{\bullet}$), superoxide radical anions ($O_2^{\bullet-}$), and free holes on the surface of the substrate (Ma *et al.* 2008).

Fe_2O_3 was also utilized at various conditions to evaluate its possible effect on lignin oxidation and ability to offer pretreatment of WS. The ability of this catalyst to augment methane production without UV irradiation was negligible *versus* the control sets. Only 240 ± 10 mL CH_4/g VS of methane was produced when there was no catalyst and no UV irradiation used (*i.e.*, double negative control). The UV light alone could only produce nonsignificant ($p > 0.05$) results for methane production (235, 247, and 235 NmL CH_4/g VS after 60, 120, and 180 min respectively). The highest amount of methane produced was after 180 min UV irradiation with the 4% Fe_2O_3 catalyst (*i.e.*, 363 ± 14 NmL CH_4/g VS; 48% increase), whereas the production was somewhat lower after 120 min UV irradiation (*i.e.*, 339 ± 16 NmL CH_4/g VS).

A major difference was observed when both the time of UV exposure was increased and the amount of Fe_2O_3 catalyst applied. This reflected the good optical properties of Fe_2O_3 compared to TiO_2 and ZnO. More electrons are released from Fe_2O_3 during the oxidation process compared to TiO_2 or ZnO, which may create more free radicals to oxidize the biomass substrate (Wong *et al.* 2016). The SEM comparisons and VA analyses confirmed that more of the WS surface was modified using Fe_2O_3 catalyst with UV exposure when compared to either TiO_2 or ZnO used with UV irradiation. This resulted in more lignin being released to be further oxidized into VA. It is well known that the fraction of lignin that escapes *via* photocatalysis is oxidized to various aromatic organic compounds (Li *et al.* 2016).

The WS pretreatment with 4% CuO and 180 min UV irradiation resulted in a BMP assay of 384 ± 16 NmL CH_4/g VS (*i.e.*, 57% increase). This was the highest amount of methane observed for all the pretreatments examined in this study. These conditions also resulted in the highest values of VA production (*i.e.*, 4.32 ± 0.15 mg VA/g VS) (Fig. 4d), as well as the most modifications to the WS surface (Fig. 3f). More modifications to the WS surface resulted in more lignin released from the substrate that could be further oxidized into VA. Kang and Kim (2012) reported that lignin oxidation results in the formation of VA, ferulic acid, and other VFAs that may be converted into VA during longer periods of UV irradiation. The general trend observed for all metal oxide photocatalysts was that production increased when the concentration of catalyst and the UV irradiation time were both increased. Hence, the results indicated that either UV exposure or metal oxide catalyst alone were not able to produce a high amount of methane. In all the experimental sets, only pretreatments using 4% catalyst and 180 min UV irradiation produced the highest methane yields. The process of the pretreatment used indicates the input of electrical energy, fresh water and feedstock. But the final products such as vanillic acid, methane and bio fertilizer from anaerobic digester can make this process industrially feasible.

CONCLUSIONS

1. The overall results observed from this study revealed that CuO was the most effective UV photocatalyst tested for the pretreatment of WS to release more lignin.
2. The maximum value of VA produced during this study was 4.32 ± 0.15 mg VA/g VS and the methane production was 384 ± 16 NmL CH_4/g VS. The BMP assay results revealed a maximum 28% increase in biodegradability and a 57% increase in methane production.
3. The use of either a metal oxide catalyst or UV irradiation alone resulted in ineffective WS pretreatment.
4. The products of lignin oxidation, such as VA that is used as vanilla flavoring agent (Sinha et al. 2008) , could be further utilized in the food industry as flavor if it could be produced in bulk.
5. The current study demonstrated a sustainable and a non-toxic pretreatment method for preparing biomass, such as WS, for further processing into bioproducts.

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