Carbon dioxide anion radical as a tool to enhance lignin valorization

Mazarji, Mahmoud; Kuthiala, Sidhant; Tsapekos, Panagiotis; Alvarado-Morales, Merlin; Angelidaki, Irini

Published in:
Science of the Total Environment

Link to article, DOI:
[10.1016/j.scitotenv.2019.05.102](https://doi.org/10.1016/j.scitotenv.2019.05.102)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Mazarji, M., Kuthiala, S., Tsapekos, P., Alvarado-Morales, M., & Angelidaki, I. (2019). Carbon dioxide anion radical as a tool to enhance lignin valorization. *Science of the Total Environment*, 682, 47-58.
<https://doi.org/10.1016/j.scitotenv.2019.05.102>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Carbon dioxide anion radical as a tool to enhance lignin valorization

Mahmoud Mazarji^{a,b}, Sidhant Kuthiala^a, Panagiotis Tsapekos^a, Merlin Alvarado-Morales^a,
Irimi Angelidaki^{a,*}

^aDepartment of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, DK-2800,
Denmark

^bSchool of Environment, College of Engineering, University of Tehran, Tehran, Iran

Abstract

Lignin is particularly recalcitrant for valorization *via* the existing pretreatment methods due to its complex cross-linking polymeric network. The aim of this study is to develop a novel integrated pretreatment strategy to exploit the potential of lignocellulosic biomass as resource for production of biofuels and aromatic chemicals. In this regard, a novel UV/TiO₂/HCOOH reaction was proposed to systematically generate hydroxyl radical ($\bullet$ OH) and carbon dioxide radical anion (CO₂ $\bullet^-$) to depolymerize lignin. Usage of 2,3-dihydrobenzofuran as a simple probe molecule showed cleavage β -O-4 linkage occurred *via* H abstraction mechanism. The addition of methyl viologen as CO₂ $\bullet^-$ scavengers proved the presence of CO₂ $\bullet^-$ in this UV/TiO₂/HCOOH reaction. Lignin and wheat straw were used to investigate the effect of different parameters, including formic acid concentration and TiO₂ dosage, on the efficiency of the reaction. At optimized conditions, the highest phenolic concentrations attained were 173.431 and 66.802 mg/g lignin and wheat straw, respectively. A cycle test was designed with the aim to favor the complete consumption of formic acid through more pretreatment cycles for producing the highest possible Total Phenolic Compounds (TPC) in the liquid phase. After the third consecutive cycle, 103.651 $\pm$ 5.964 mg-TPC/g, was obtained. Meanwhile it was found the remaining wheat straw solid fibers used for biogas production, showed 11.0% increase biogas production and increased degradation rate compared to the untreated wheat straw.

Keywords: lignin oxidation; lignocellulosic biomass; pretreatment; carbon dioxide radical anion; high-value products

25 1. Introduction

26 Biomass is attracting increased interest due to its potential as an inexpensive, sustainable, and renewable
27 carbon source for partial substitution of fossil resources (Menon and Rao, 2012). Current research is focused
28 on utilizing lignocellulosic biomass within a circular economy framework for the production of energy,
29 valuable chemicals, materials, and fuels. Lignocellulose is the major structural component of woody and
30 non-woody plants consisted of cellulose, hemicellulose, and lignin. While technologies to process cellulose
31 and hemicellulose are well developed, lignin processing constitutes a considerably more challenging task
32 (Kärkäs et al., 2016). Lignin, which constitutes 10–25 wt% of lignocellulosic biomass, is the only renewable
33 source of aromatic hydrocarbons in the biosphere exceeding 300 billion tons (Smolarski, 2012). Lignin
34 consists of three phenyl propane units, namely, p-coumaryl, coniferyl, and sinapyl alcohols connected by
35 ether and carbon-carbon bonds such as β -aryl ether (β -O-4), α -aryl ether (α -O-4), diphenyl ether (4-O-5), and
36 biphenyl (5–5) in a bulky and highly functionalised fashions (Pérez et al., 2002). If efficiently
37 depolymerized, a pallet of value-added renewable chemicals (i.e. phenols, aromatic, and aliphatic
38 compounds) can be produced to replace fossil fuel based products. However, it is difficult to depolymerize
39 lignin into various products because of its highly cross-linking and random structure (Wang et al., 2018).
40 Due to the high thermal and chemical stability of C-C and C-O bonds, lignin is resistant to traditional
41 physical, chemical, and biological degradation (Brodeur et al., 2011; Pineda and Lee, 2016). Considering
42 this, several research groups are investigating different pretreatment methods to target cleaving abundance of
43 β -O-4 or biphenyl-type (5-5) linkages to produce different possible compounds (Pineda and Lee, 2016).
44 Many methods have been proposed to realize more efficient lignin oxidative depolymerization strategies,
45 while effective conversion to stable products is still a big challenge due to uncontrollable overoxidation of
46 substrate to gaseous products (Sun et al., 2018).

47 Different strategies have been developed in order to i) generate valuable aromatic chemicals and/or provide a
48 source of low-molecular-mass feedstocks suitable for downstream processing and ii) increase biomass
49 biodegradability for bioenergy production. In this regard, advanced oxidation process associated with a
50 generation of highly reactive hydroxyl radical ($\bullet$ OH) received remarkable attention due to its ability to target

51 a wide variety of refractory organic pollutants. These pollutants including persistent organic pollutants
52 (POPs) (Dong et al., 2015) and synthetic dyes (Rauf and Ashraf, 2009) are difficult to eliminate from liquid
53 effluents with conventional methods due to their recalcitrant features. Photocatalysis, among these strategies,
54 involves generation of highly reactive radicals namely $\bullet\text{OH}$ and superoxide anion radical ($\text{O}_2^{\bullet-}$) under
55 controlled operation. Unlike other pretreatments which demand severe conditions (high temperature and
56 pressure), this pretreatment can be carried out efficiently under mild conditions to produce highly valuable
57 phenolic or other low molecular weight compounds.

58 The main drawback of a typical photocatalysis is an uncontrolled oxidation resulting in a complete
59 mineralization of the organic matter (Alvarado-Morales et al., 2017; Nair et al., 2016). Some reports
60 indicated that $\bullet\text{OH}$ is involved in the photocatalytic degradation of lignin (Li et al., 2015; Machado et al.,
61 2000), while others showed that $\text{O}_2^{\bullet-}$ plays the main role in this field (Chen et al., 2018; Gong et al., 2017).
62 The advantages of using the $\bullet\text{OH}$ contra $\text{O}_2^{\bullet-}$ for lignin depolymerization is because its redox potential is
63 higher (2.80 V, vs. standard hydrogen electrode (SHE)) compared to redox potential of $\text{O}_2^{\bullet-}$ (0.890 V, vs.
64 SHE). However, $\bullet\text{OH}$ is considered as a highly non-selective radical which often results in a complete
65 decomposition of compounds in the reaction medium (Gong et al., 2017). Moreover, the production of $\text{O}_2^{\bullet-}$
66 during the photocatalytic reaction is not controllable, since it can easily transform to hydroperoxyl radical
67 with a less redox potential. These reasons highlight the significance of introducing a new radical type in the
68 field of lignin oxidation.

69 Carbon dioxide anion radical ($\text{CO}_2^{\bullet-}$) is a very strong one-electron oxidant acting both as electron transfer
70 and H abstraction mechanism (Rosso et al., 2001) and has recently received considerable attention for
71 remediation chlorinated hydrocarbons and chromium contamination (Gu et al., 2017; Ren et al., 2017). In
72 most cases, various methods, including vacuum ultraviolet (UV) irradiation (i.e. 175 nm) of aqueous
73 solutions containing formate at pH 4 (Rosso et al., 2001), γ radiolysis of water (and water solutions) in air
74 (Todres, 2009), thermally activated persulfate system (50 °C) in the presence of formate anions (Ren et al.,
75 2017), and activation of formic acid and sulfate radical produced under UV/ $\text{S}_2\text{O}_8^{2-}$ process (Gu et al., 2017)
76 are applied to generate $\text{CO}_2^{\bullet-}$. $\text{CO}_2^{\bullet-}$ is characterized by better electron transfer and H abstraction

mechanisms -compared to $O_2^{\bullet-}$ (Rosso et al., 2001). It is well known that pure lignin and lignin rich-biomasses contain β -O-4 linkages as the weakest available bond, so the cleavage of C_{aryl} -O bonds in lignin is a facile alternative approach for lignin depolymerization (Zhang et al., 2016). The reaction of $CO_2^{\bullet-}$ with β -O-4 linkages in lignin would lead to the formation phenolic monomers.

The present study examines a novel advanced oxidation route using UV/TiO₂/HCOOH for depolymerization of lignin into phenolic compounds by generation of newly $CO_2^{\bullet-}$ alongside powerfully $\bullet OH$ at a very mild conditions. Taking into account the pure lignin and lignin rich-biomasses contain β -O-4 linkages as the weakest available bond, so the cleavage of C_{aryl} -O bonds by H abstraction would present a simple and facile alternative approach for lignin depolymerization. Production of $CO_2^{\bullet-}$ and $\bullet OH$ for cleavage of the β -O-4 linkage, initially was tested against 2,3-dihydrobenzofuran as a simple probe molecule. Then, the existence of $CO_2^{\bullet-}$ in the system was examined by using methyl viologen. After detailed mechanism analysis, the proposed process was adopted to test the applicability of the pretreatment method for the production of high-value products from lignin and wheat straw as an actual lignocellulosic biomass. In addition, the pretreated materials were anaerobically digested to evaluate the suitability of the photocatalytic solution for energy recovery purposes.

2. Materials and methods

2.1. Materials

Sigma-Aldrich Kraft lignin (product number 370959), abbreviated as lignin and formic acid (analytical grade, ACS reagent, $\geq 98\%$) were purchased from Sigma-Aldrich while gases were provided by AGA A/S (Copenhagen, Denmark). Photocatalyst grade Titanium (IV) Oxide (TiO₂) (product number 248576) composed of anatase and $\sim 5\%$ rutile in a powder form – 325 mesh, $\geq 99\%$ trace metal basis was obtained from Sigma-Aldrich. Wheat straw residues were collected from the surrounding area of Copenhagen (Zealand, Denmark) and cut to less than 0.50 cm length by a cutting mill machine (SM 200, Retsch GmbH, Germany). Subsequently, the resulting fibers were sieved using 1 mm sieve (size 18 mesh) to obtain a homogeneous particle size. The total solids (TS) and volatile solids (VS) of the wheat straw were $92.80 \pm$

102 0.40% and $86.70 \pm 0.10\%$ of fresh matter. The more detailed characterizations can be found elsewhere
103 (Alvarado-Morales et al., 2017).

104 2.2. Experimental procedure

105 Photocatalytic batch experiments were conducted in a quasi-collimated beam apparatus. A doped medium
106 pressure lamp (SR HUV700 (European patent: EP1463091A2), 700 W, Scan Research A/S, Denmark) was
107 used inside the reactor setup with a specific feature of enhancing emission at low wavelength. Detailed
108 characteristics of the lamp and reactor setup were described previously (Hansen et al., 2013). Prior to the
109 experiments, preparation started with overnight soaking samples including lignin and wheat straw in an
110 appropriate volume of formic acid (98-100 w/w%) solution. After 24 h soaking of the samples, distilled
111 water was added to the solution to obtain the desired concentration of formic acid at 100 mL solution.
112 Equilibration period at dark was initiated with addition of the desired amount of TiO_2 into the resulting
113 solution in order to establish an adsorption-desorption equilibrium among the photocatalyst surface and
114 sample's molecules. After 60 min of adsorption, the UV lamp was turned on, followed by air sparging.
115 Aquarium air pump with flow rate of 1.5 L/min was used to assure the availability of sufficient amount of
116 oxygen and removal of other gasses generated during the reaction from the reactor. At given intervals of
117 illumination, the samples of reaction solution were collected and centrifuged for 10 min at 13000 rpm. The
118 supernatant was collected for determination of total phenolic compounds (TPC) % change and soluble
119 chemical oxygen demand (sCOD) % change. The solid residues were collected by passing the solution
120 through a Whatman® 41 (125 mm diameter) filter paper. After recovering of solid residues by the filter
121 paper, the leftover was dried completely in an oven at 60 °C for further experiments and characterizations.

122 2.3. Experimental design

123 The experiment was built according to the response surface methodology (RSM) for two independent
124 variables namely formic acid concentration (x_1) and TiO_2 dosage (x_2). The design chosen for the study was a
125 faced central composite design (fCCD), where the independent variables were investigated at three levels of
126 $(-1, 0, +1)$. Preliminary experiments were conducted to determine a narrower range of independent variables

127 prior to designing the experimental runs. For this purpose, experiments were carried out by varying a single
 128 factor while keeping all other factors fixed at a specific set of conditions. It should be noted that due to the
 129 results of preliminary experiments, the experimental ranges for formic acid concentration and TiO₂ dosage
 130 were not similar for lignin and wheat straw sample. According to the obtained experimental data, the ranges
 131 of independent variables and experimental derived from fCCD are given in Table 1.

132 For statistical calculations and model developments, the real values of the independent variables (X_i) were
 133 converted to dimensionless coded values between +1 and -1 (x_i) according to Eq. (1):

$$x_i = \frac{X_i - X_0}{\delta X} \quad (1)$$

134 where X_0 and δX are the real value of X_i at the center point and the step change, respectively.

135 In total, 10 experiments for each case, comprising of 4 factorial points, 4 axial points, and 2 replications at
 136 the center points were performed for allowing the estimation of experimental error. Table 2 provides the
 137 details of the 10 experimental conditions planned for each experiment.

138 Experimental data were analyzed using statistical analysis software JMP Pro 13 (SAS Institute Inc.) and
 139 fitted to a second-order polynomial model to optimize the variables in the oxidation process. The quadratic
 140 equation model for predicting the optimal conditions as a function of the two independent variables can be
 141 expressed as Eq. (2):

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{i=1}^n \sum_{j=i+1}^n \beta_{ij} x_i x_j \quad (2)$$

142 where, Y (%) represents the predicted response; x_i and x_j are the coded i and j^{th} independent coded variable;
 143 β_0 , β_i , β_{ii} , and β_{ij} are the intercept, the first-order model coefficient, the quadratic coefficient of variable i , and
 144 the interaction coefficient of variables i and j , respectively. The reliability of the fitted model was evaluated
 145 based on the analysis of variance (ANOVA) and as well as the coefficient of determination (R^2).

146 After developing the second-order polynomial model, the optimal values of the parameters were achieved by
 147 analyzing the surface and counter plots by solving the obtained regression equations. For the lignin sample,
 148 the goal was set to maximize the TPC % change. For wheat straw, the optimization was focused on

149 maximum TPC % change and minimum sCOD % change. This is explained as: i) high-value products are
150 obtained in the liquid phase by high TPC, ii) less solubilization of wheat straw fibers is achieved into the
151 solution (i.e. the treated wheat straw can be used for biogas production, hence sCOD amount in solution is to
152 be limited).

153 2.4. Mechanism study by probe molecule and radical scavenger

154 In order to investigate the underlying mechanism behind the oxidation process of lignin, 2,3-
155 dihydrobenzofuran as a simple probe molecule was used. At the optimal conditions achieved for lignin, the
156 reaction was carried out in a 100 mL solution containing 0.2 g/L of 2,3-dihydrobenzofuran, 0.1 g/L TiO₂, and
157 0.25 M formic acid. Then, the reaction solution was aerated under vigorous stirring. At specific times
158 samples were collected. Before storage, the samples were firstly centrifuged for 10 min at 13000 rpm and
159 then, the recovered supernatant was filtered by a 0.45µm filter (Agilent®). The control experiment without
160 formic acid was also conducted at same conditions to study the effect of photocatalytic oxidation of TiO₂. To
161 prove the CO₂•⁻ formation responsible for the oxidation of lignin, methyl viologen dichloride (MV²⁺, 98%,
162 Sigma-Aldrich) was introduced to the system. This method was similar to the above method of
163 photocatalytic activity test except addition of 0.10 and 0.01 mM scavenger in the presence of lignin
164 according to the method presented by (Ren et al., 2017).

165 2.5. Cycling test and biochemical methane potential (BMP) assays

166 After selection of optimized conditions, the cycle test was designed to achieve the highest concentration of
167 value added chemicals (i.e. phenolics) in the liquid phase. At the end of each cycle wheat straw fibers were
168 separated from the solution. In this regard, the first cycle (denoted as 1st cycle) was run for 240 min after
169 which the solution was passed through filter paper to separate the solid and liquid phase. The liquid phase
170 was then reused for the second and third cycle adding always fresh 1 g/L wheat straw. The final collected
171 treated wheat straw after production of phenolics was dried in an oven at 60 °C for 24 hours and was used for
172 the methane production.

173 BioMethane potential (BMP) assay was performed to determine biodegradability and methane production
 174 potential of the pretreated sample after 1st, 2nd cycle, and untreated wheat straw. Samples were prepared
 175 according to the guidelines of Angelidaki et al (Angelidaki et al., 2011). All samples were prepared in
 176 triplicate in 337 mL glass bottles at an organic load of 1.50 gVS/L. Avicel (PH-101, Sigma Aldrich) was
 177 used as the control at the same organic load. Blank bottles containing inoculum and water were also prepared
 178 to define the background methanation from the inoculum. The inoculum was obtained from the effluent of a
 179 well-performing lab-scale biogas reactor fed with source separated municipal organic waste. Before usage,
 180 the inoculum was placed in a thermophilic incubator (55±1°C) to be degassed for 7 days to eliminate the
 181 background methane production. During the incubation period, the reactors were shaken once a day to avoid
 182 the development of dead zones. The methane concentration in the headspace of batch reactors was
 183 determined using a gas chromatograph (Thermo Scientific™, Trace™ 1310) as previously described
 184 (Khoshnevisan et al., 2018). For the degradability experiments, the methane yields were reported at standard
 185 temperature and pressure (STP) conditions. The experiments were conducted at thermophilic conditions
 186 (54±1°C).

187 **2.6. Analytical methods and calculations**

188 **2.6.1. Characterization of aqueous phase products**

189 The production of phenolics was probed on a spectrophotometer (UV–vis spectrophotometer UH5300
 190 Hitachi) over the wavelength ranges of 250 to 400 nm. The concentration of total phenolic compound (TPC)
 191 was determined based on the Folin-Ciocalteu method (Ainsworth and Gillespie, 2007). A characteristic peak
 192 at 725 nm reveals the TPC content after the reaction with the Folin-Ciocalteu reagent and sodium carbonate.
 193 A calibration curve for each measurement was done using gallic acid as standard and subsequently, a
 194 calibration plot was constructed to quantify the TPC content. TPC was expressed as mg phenolics produced
 195 per g of lignin used (mg/g) depending on the lignin content of the substrate. TPC was calculated using the
 196 equation as follows:

$$TPC = \frac{[C] \times [V]}{[M] \times [Lignin\ content]} \quad (3)$$

197 where M, V, and C are loading of wheat straw or lignin (g/L), volume of solution (L), and quantified
198 concentration of phenolics from calibration curve (mg/L), respectively. The lignin content value was $26.70 \pm$
199 2.40 %TS and 100% for wheat straw and lignin sample, respectively (Tsapekos et al., 2017).

200 COD was determined according to the Standard Methods (APHA, 1998). For the soluble COD (sCOD), the
201 samples were centrifuged and only the supernatant was used. TPC or sCOD (%) change was determined
202 using the following equation:

$$Y (\%) \text{ change} = \left[\frac{(C_t - C_0)}{C_0} \right] \times 100 \quad (4)$$

203 where C_t and C_0 are the concentration of TPC (mg/g) or sCOD (mg/L) at time (t) and initial time,
204 respectively.

205 Analysis of the molecules in the liquid phase produced after the process was conducted with a gas
206 chromatography/Mass spectrometry (GC/MS, Agilent 7890A/ 5975C). The analysis conditions were chosen
207 as follow: a capillary column was used (HP-INNOWAX, 30 m $\times$ 0.25 mm $\times$ 0.25 μ m) at programmed oven
208 temperature of 60 °C, hold for 2 minutes at 60 °C and then ramped up at a rate of 10 °C/minute to 260 °C
209 and hold at this level for extra 10 minutes; the injector was kept at 280 °C in spit mode with spit ratio of 1:10
210 and splitless; helium was used as the carrier gas. The listed compounds were identified by comparing with
211 National Institute of Standards and Technology (NIST) mass spectral database.

212 Formate and formic acid were detected and quantified using high performance liquid chromatography,
213 HPLC. The HPLC (Agilent) had a refractive index detector (detection of sugars, VFAs and ethanol) and a
214 Bio-Rad Aminex HPX-87H column (300 mm $\times$ 7.8 mm) with 0.04 M H₂SO₄ as eluent and flow rate was set
215 to 0.6 mL min⁻¹ with column oven temperature set to 63.5 °C.

216 **2.6.2. Characterization of solid phase fibers**

217 The element measurement of non-volatile product was carried out on an elemental analyzer (Elementar vario
218 MACRO cube). Crystallinity of the wheat straw samples was determined by X-ray diffraction (XRD) using a

219 diffractometer (X'Pert Pro, Panalytical) operated at 40 mA and 45 kV. The crystallinity index (CI) was
220 estimated based on the following equation described by (Segal et al., 1959):

$$CI(\%) = \left[\frac{(I_{002} - I_{18})}{I_{002}} \right] \times 100 \quad (5)$$

221 where I_{002} is the intensity of the diffraction at 002 peak position ($2\theta \approx 22.5^\circ$) for cellulose and I_{18} is the peak
222 for the amorphous region at about $2\theta \approx 18^\circ$.

223 The functional groups were measured by Fourier transform infrared spectroscopy (FTIR) using a Spectrum
224 One spectrophotometer (Nicolet iS50, Thermo Fisher Scientific). The total crystallinity Index (TCI) was
225 calculated according to the method of Nelson and O'Connor (Nelson and O'Connor, 1964), i.e. ratio between
226 the peaks at 1372 and 2920 cm^{-1} from FTIR spectra. Scanning electron microscopy (SEM) analysis was
227 performed with an FEI Quanta 200 ESEM FEG without any coating to study morphological change for the
228 treated wheat straw after the process. For a separation of the pretreated solid fibers, a glass microfiber filter
229 (Whatman GF/C 470) was used. The treated and untreated wheat straw samples were dried in an oven at 100
230 $^\circ\text{C}$ for 24 hours prior to characterization.

231 2.6.3. Statistical analysis

232 One-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference test (LSD, $p <$
233 0.05) was conducted to verify the existence of a statistically significant difference among experimental
234 measurements. All statistical analyses were performed using OriginPro 9.0.0 SR2 software (OriginLab
235 Corporation, USA).

236 3. Results and discussion

237 3.1. Mechanism analysis

238 3.1.1. Mechanism formulation and proposed reaction pathway

239 The used lamp is capable of homolysis of water since it can irradiate the wavelength in the range of 150 to
240 200 nm (Zoschke et al., 2014), and water molecule can be converted to $\bullet\text{O OH}$ and H atom ($\text{H}\bullet$). Then, $\bullet\text{OH}$
241 and $\text{H}\bullet$ can react with formate or formic acid to produce $\text{CO}_2\bullet^-$ (Rosso et al., 2001). This mechanism of

242 $\text{CO}_2^{\bullet-}$ production can be expressed as the chain of reactions through the multistep continuous reactions (Eqs.
 243 (6) – (10))

244 The presence of TiO_2 can promote the production of $\bullet OH$ in the solution. The following mechanism is
 245 proposed for UV/ TiO_2 system. The photoinduced electrons and holes were generated upon excitation of
 246 TiO_2 , as described in Eq. (11). These separated electrons would subsequently eject on the surface of TiO_2 ,
 247 ready for quenching by O_2 to form $\text{O}_2^{\bullet-}$ by Eq. (12). Furthermore, $\bullet OH$ would generate from oxidation of
 248 hydroxyl anion at the holes (Eq. (13)).

249 The combination of photocatalysis (the direct formation of $\bullet OH$ and $\text{O}_2^{\bullet-}$) and photolysis process (the direct
 250 formation $\text{CO}_2^{\bullet-}$) can establish advanced oxidation conditions comparable to other traditional advanced
 251 oxidation processes (AOPs).

252 An initial understanding of the reaction mechanisms was attempted by using a simple molecule having β -O-4
 253 linkage, similar to the lignin molecule. Specifically, 2,3-dihydrobenzofuran was used as a probe molecule
 254 (i.e. representative of β -O-4 linkage inside the lignin) in order to elucidate the oxidation mechanism of $\text{CO}_2^{\bullet-}$
 255 and $\bullet OH$. The obtained reaction products were determined by GC/MS and a brief reaction pathway of 2,3-
 256 dihydrobenzofuran oxidation in the presence of formic acid and/or TiO_2 was proposed. As shown in Fig. 1,
 257 after 30 min, 2,3-dihydrobenzofuran converted to benzofuran and 2-methoxybenzaldehyde with approximate
 258 conversion of 39.0% and 67.0%, respectively. With the extension of reaction time, the selectivity of
 259 benzofuran decreased, vice versa the selectivity of 2-methoxybenzaldehyde increased. Hence, it can be

concluded that the reaction driven by $\text{CO}_2^{\bullet-}$ led to the selective attack of β -O-4 linkage *via* H abstraction from aromatic ring of 2,3-dihydrobenzofuran (to form benzofuran), as well as a competing pathway involved oxygen addition to carbon-centered radical, elimination to form aldehyde accompanying with cleavage of furan ring, which ultimately resulted in the production of 2-methoxybenzaldehyde. Furthermore, the presence of TiO_2 would catalyze this step to produce more of $\text{CO}_2^{\bullet-}$ (Eqs. (7) and (8)), besides the eventual production of $\bullet\text{OH}$. An analogous experiment was also conducted in the presence of only formic acid, and the similar results were obtained with lower conversion yield. This implies the necessity of using TiO_2 for enhancement of overall conversion yield. However, it should be mentioned during oxidation of the probe molecule at the presence of only TiO_2 , the formation of $\bullet\text{OH}$ and $\text{O}_2^{\bullet-}$ led to the unselective direct degradation of the probe molecule (data not shown).

Fig. 1. Mole fraction profiles of 2,3-dihydrobenzofuran oxidation and its obtained products as a function of time. Conditions: 2,3-dihydrobenzofuran loading 0.2 g/L; formic acid concentration 0.25 M; TiO_2 dosage 0.1 g/L; final volume 100 mL; saturated with air.

3.1.2. Radical identification

Mainly electron spin resonance (ESR) and radical scavenger experiments are employed for identification of $\text{CO}_2^{\bullet-}$. In this context, using ESR experiment to verify the presence $\text{CO}_2^{\bullet-}$ and $\text{SO}_4^{\bullet-}$ was reported by (Ren et al., 2017). In addition, methyl viologen (MV^{2+}) has been widely used in the literature (Berkovic et al., 2012,

2010; Gu et al., 2017), as a competitive scavenger to react with $\text{CO}_2^{\bullet-}$. Hence, this method was employed to identify the existence of $\text{CO}_2^{\bullet-}$. Fig. 2 shows the inhibitory effect of this scavenger relatively to control experiment ran at the center point conditions for lignin. It can be seen from Fig. 2 that TPC concentration in the absence of MV^{2+} increased to 164.112 ± 0.991 mg/g after 180 min. TPC production in the presence of 0.01 and 0.10 mM MV^{2+} were decreased to 130.330 ± 1.164 and 120.512 ± 1.092 mg/g, respectively. The results showed that the reaction was quenched with the addition of $\text{CO}_2^{\bullet-}$ scavenger as described by Eq. (14). Therefore, $\text{CO}_2^{\bullet-}$ as the predominant active species is responsible for the TPC production in the UV/ TiO_2 /HCOOH reaction.

286

287 Fig. 2. TPC concentration (mg/g) as a function of time with the addition of different methyl viologen (MV^{2+})
 288 concentration at center point (experiments 6–7). Fixed experimental conditions: lignin loading 0.2 g/L; final
 289 volume 100 mL; saturated with air.

290 3.2. Effect of parameters on TPC results of lignin and wheat straw

291 The results from the first optimization step (fCCD) are presented in Fig. 3. The yield in terms of TPC for
 292 each experimental run is also reported in Table 2. For the simplicity of explanation, the experiments were
 293 divided into three categories based on the level of formic acid concentration (x_1): experiments 1-3, 4-7, and
 294 8-10 where the level of formic acid concentration was set to the value of +1, 0, and -1, respectively.

Experiments 1-3 were done using the highest formic concentration while the dosage of TiO₂ varied from the lowest level to the highest one. As can be seen from experiment 1 to 3 in Fig. 3a for lignin samples, the TPC production increased by increasing the TiO₂ dosage; highlighting the importance of an adequate photocatalyst concentration. This observation is in agreement with other studies (Gong et al., 2017; Kansal et al., 2008; Ma et al., 2008; Nair et al., 2016). In contrast, the increase of the TiO₂ during wheat straw treatment was connected with decreased TPC production as shown in Fig. 3b. In addition, no appreciable changes can be found among experiment 1 (photocatalyst-free condition) and 2 (medium level of the photocatalyst used). This can be attributed to the fact that an uncontrolled photocatalysis using TiO₂ would favor the attack on other parts of wheat straw (i.e. cellulose or hemicellulose) which does not result in the production of phenolics.

For the second category (i.e. experiments 4-7 with the formic acid concentration at the level of 0), the increased TiO₂ dosage initially led to increased TPC production to reach the highest (experiment 5) and subsequently, decreased at experiments 6 and 7 (denoted as center point in Fig. 3a). On the other hand, considering the same level experiments for wheat straw, no significant change occurred. Experiments 8, 9, and 10 where lignin or wheat straw was soaked in water without addition of any formic acid (level of -1 for formic acid concentration) showed the lowest production of TPC or even negative in the case of lignin. This confirms a role of formic acid in combination with TiO₂ for an improved TPC in the entire process. This result is in line with the probe molecule experiments when no formic acid was used. At this condition, unselective direct degradation of the probe molecule happened. It was also observed that by further increasing TiO₂ dosage, less TPC was produced for lignin sample. Thus, lignin would be mineralized due to harsher conditions provided by presence of TiO₂ in a short manner of time, when no formic acid was used. In the case of wheat straw, the opposite effect of lignin samples was achieved (Fig. 3b). Particularly, in these experimental conditions, a slight increase in the TPC production was achieved by an increment of TiO₂ dosage. This enhancement can be due to the direct effect of mediated radicals from TiO₂ which agrees well with our previous study (Alvarado-Morales et al., 2017). Despite the decrease in TPC for lignin at experiments 8-9, it was observed that TPC for wheat straw was increased for these experiments. This could

321 be due to the fact that the provided harsher conditions did not only exerted a strong oxidation action for
322 phenolics production by oxidation of lignin component. The radicals would favor the attack on other parts of
323 wheat straw which does not result in the production of phenolics. To study the effect of radicals on cellulose
324 or hemicellulose of wheat straw, sCOD % change was introduced at the next step during model
325 development. For experiment 10 (control; only water), no change or slightly higher production of TPC for
326 lignin and wheat straw, respectively were found, which is in line with our previous study (Tsapekos et al.,
327 2018).

328 Fig. S1 presents the time evolution of UV-vis spectra for the experiment ran at the center point conditions for
329 lignin and wheat straw samples. For both samples, the peak corresponding to phenolics at 280 nm increased
330 with an extension UV illumination time. Increased amount of phenolics was produced in the case of lignin
331 compared to the wheat straw sample. For wheat straw, there was no appreciable increase in the concentration
332 of phenolic compounds in the aqueous phase could be observed. The reason for this observation could be due
333 to the lower limited content of lignin in wheat straw (i.e. 26.7 ± 2.4 %TS). It is interesting to note that
334 phenolic production continuously increased with irradiation time, suggesting that overoxidation of the
335 produced phenolics to CO₂ and H₂O did not occur. Based on the conducted experiments, the TPC production
336 % change after 180 min for lignin and 240 min for wheat straw were chosen for model development.

Fig. 3. TPC concentration (mg/g) as a function of time under different conditions according to the fCCD for a) lignin and b) wheat straw. Center point corresponds to the average of experiments 6–7. Fixed experimental conditions: lignin loading 0.2 g/L; wheat straw loading 1 g/L; final volume 100 mL; saturated with air.

3.3. Model development and validation ANOVA

A fCCD design, of 10 experiments, was run in order to explore the effects of two independent variables on the responses in a systematic way. Two models were constructed based on the experimental results of the fCCD, namely Eqs. (15) and (16). In the case of wheat straw, an additional model was built based on sCOD % change expressed in the Eq. (17). The actual responses observed from running experiments are listed in Table 2. The final mathematical models associated with the responses were obtained after elimination of insignificant terms ($p > 0.05$). The final models for predicting the TPC and sCOD % change for treated lignin and wheat straw in terms of coded variables (x_1 : Formic acid and x_2 : TiO_2) are:

$$\text{TPC of lignin \% change} = 51.87 + 20.07 \times x_1 + 4.65 \times x_2 + 15.99 \times x_1 \times x_2 - 40.76 \times x_1^2 \quad (15)$$

$$\text{TPC of wheat straw \% change} = 70.50 + 25.14 \times x_1 - 4.85 \times x_1 \times x_2 - 27.06 \times x_1^2 \quad (16)$$

$$\text{sCOD of wheat straw \% change} = 55.30 - 38.67 \times x_1 + 13.83 \times x_1^2 - 15.56 \times x_2^2 \quad (17)$$

The adequacy of the model was justified through ANOVA and the results are shown in Table S1. The models constructed were highly significant ($p \leq 0.0005$) and the test of lack-of-fit was satisfactory insignificant. Furthermore, the second-order polynomial models were highly significant for 95% confidence level as shown by the F -ratios of 111.543 and 167.732 for TPC % change of lignin and wheat straw, respectively. F -ratio was found to be 41.244 for sCOD % change. The fitted model was further evaluated by the correlation coefficient (R^2). According to the ANOVA results (Table S1), R^2 values were in good agreement with the adjusted R^2 , indicating that the effects included in the models are sufficient for modeling the responses. All predictive models presented in this study can be used for estimation of the TPC and sCOD % change within the ranges of the experimental region.

3.4. Response surface plotting for evaluation of operational parameters and optimization of the process

Fig. 4 presents the response surface graphs which are the graphical representation of the regression equations Eqs. (15) - (17) values within the levels $[-1, +1]$. The response surface graph of Eq. (15) is presented in Fig. 4a, where the predicted TPC % change is plotted as a function of the formic acid concentration and TiO_2 dosage in the coded form. As shown, the TPC % change increased with increasing formic acid concentration. The formic acid concentration appears to have had the strongest effect on TPC change of lignin, which is also reflected in Eq. (15), as the magnitude of the coefficient in front of x_1 has the highest value with a positive sign. The maximum TPC change for lignin ($\approx 62\%$) was observed in the level of formic acid from 0.14 to 0.80 while TiO_2 dosage from 0.61 to 0.96; and, x_1 resulted in low TPC change when set at the maximum or minimum level of the experimental range. Further increase of formic acid concentration from 0.80 to 1 coincided with depletion in TPC % change. This observation indicates that under these conditions, the amount of $\bullet\text{OH}$ and $\text{H}\bullet$ formed by water homolysis (Eq. (6)) is inadequate to react with formate or formic acid to produce $\text{CO}_2^{\bullet-}$ (Eqs. (7) – (10)). Thus, the $\text{CO}_2^{\bullet-}$ formation became independent of formic acid concentration. All of these effects are expressed in the model by the quadratic term of this parameter (x_1^2).

Optimization of the input space of the reaction (i.e. Eq. (15)) was obtained with the highest TPC increase of $64.0 \pm 4.121\%$ at the conditions as follows: formic acid concentration = 0.4 M ($x_1 = +0.33$) and TiO_2 dosage = 0.2 g/L ($x_2 = +1$). The validation of predicted optimized conditions was done by conducting the experiment at the aforementioned conditions. Indeed, the comparison between the predictive ($64.0 \pm 4.121\%$) and experimental results ($63.420 \pm 3.511\%$) had a very good agreement as they did not differ significantly. The increase obtained experimentally corresponded to 173.431 ± 2.970 mg/g TPC with the starting TPC concentration of 106.40 ± 0.33 mg/g.

The effects of x_1 and x_2 on the TPC % change of wheat straw is demonstrated in Fig. 4b; which is the graphical representation of Eq. (16). As shown, by increasing x_1 , the TPC % increased almost irrespectively of x_2 . As indicated in Eqs. (7) – (8), $\bullet\text{OH}$ availability in the presence of formate or formic acid, enhanced the formation of $\text{CO}_2^{\bullet-}$. As mentioned above, the lamp itself is capable of production of $\bullet\text{OH}$ and $\text{H}^\bullet$ and on top of this, the presence of TiO_2 provided the additional source for $\bullet\text{OH}$ (Eq. (13)). In this regard, the increase of x_2 (TiO_2 dosage) affected the reaction insignificantly. This implies the independence of TPC change from the addition of TiO_2 . Moreover, TPC change had a rapid increase with increasing x_1 within the level of -1 to 0 . Subsequently, the further increase of x_1 (0 to 1) had no significant change in the reactivity, indicating that there was an optimum concentration of formic acid. In other words, x_1 played an influential role when its level declined to 0 and less. As it discussed earlier in Eqs. (8) – (9), the increase in formic acid concentration has a positive effect on the production of $\text{CO}_2^{\bullet-}$, thus contributing directly to the increase of TPC. Fig. 4c presents the effect of x_1 and x_2 on the sCOD % change of wheat straw resulting from Eq. (17). The response was plotted as a function of two factors varying in the factorial part of the design $[-1, +1]$. It was observed that that highest sCOD % change ($\approx 106\%$) was obtained at low x_1 and middle x_2 . In the real value format, sCOD % decreased with the increase in formic acid concentration, while sCOD % change increased with increase in TiO_2 up to 0.05 g/L after which continuous decrease was observed. It is interesting to note that, sCOD% change (Fig. 4c) showed an irreversible trend with TPC% change (Fig. 4b). This proposes, the sCOD serves as an indirect measurement of TPC: the increase in solubilized particulate matter % would result in the decrease in TPC %. In other words, the solubilization of particulate matter must be kept low if

the TPC increase is taken into consideration. To this end, the experimental conditions must be set at the highest level of x_1 and low or high values of x_2 to minimize sCOD % change. It is important to note that setting the value of x_2 at the low level (i.e. photocatalyst-free condition) is more beneficial compared to the highest level of x_2 which involves the additional cost of separation for the used photocatalyst. According to Figs. 4b and 4c, the optimal conditions of the pretreatment of wheat straw fibers for maximum TPC and minimum sCOD % change corresponded to 0.35 M formic acid ($x_1 = +0.75$) and photocatalyst-free condition ($x_2 = -1$). The prediction of Eq. (16) for the TPC change under these conditions, corresponds to $77.0 \pm 2.893\%$, and based on Eq. (17) for to a $17.0 \pm 7.734\%$ increase of sCOD. Finally, these conditions were verified experimentally, suggesting the good agreement between the predicted and observed ($75.822 \pm 3.071\%$ for TPC and $19.212 \pm 5.341\%$ for sCOD) values. The treated wheat straw solution at the optimized conditions contained 66.802 ± 2.281 mg/g TPC.

Fig. 4. Surface plots of a) TPC % change as a function of formic acid concentration (x_1) and TiO_2 dosage (x_2) at irradiation time of 180 min for lignin, and surface plots of b) TPC % change, and c) sCOD % change as a function of formic acid concentration (x_1) and TiO_2 dosage (x_2) at irradiation time of 240 min for wheat straw. [Fixed experimental conditions: lignin loading 0.2 g/L; wheat straw loading 1 g/L; final volume 100 mL; saturated with air].

3.5. Characterization of optimized conditions

3.5.1. Qualification of the liquid phase

Phenolics produced at the optimized conditions were qualified and the GC/MS total ion chromatograms are shown in Fig. 5 for both lignin and wheat straw samples. The corresponding retention time, molecular

weight, and other identifiable compounds are listed in Table 3, in which their corresponding charge-to-mass ratios (m/z) are also listed. In the case of lignin, the main reaction products were guaiacol, 2-ethylphenol, and vanillin. The presence of 2-ethylphenol and vanillin demonstrates the occurrence of oxidation during the photocatalytic reaction, which is in agreement with literature (Chen et al., 2018; Gong et al., 2017; Nair et al., 2016). Based on experimental and product characterization results together with a bond dissociation energy (BDE) of major bonds within lignin inter unit linkage from literature, a possible lignin decomposing pathway is proposed for lignin oxidation. In summary, depolymerization of lignin led to production of phenolics as the major product. This is supported by the fact that all the products detected were phenolics (e.g. guaiacol, 2-ethylphenol, and vanillin). The identity of these products was found to support the H abstraction pathway, as illustrated in the degradation pathway of probe molecule in the section of 3.1.1. It is likely that the most pronounced reactions in the case of lignin are degradation *via* β -O-4 and C_α - C_β , scission reactions. It could be due to the fact that the lowest and second lowest of BDE among linkages in lignin are C_β -O-4 (245.3 kJ/mol) and C_α - C_β (259.2 kJ/mol), indicating these bonds are relatively easier to break during the oxidation process (Feng et al., 2019).

Diverse peaks were also detected for the treated wheat straw (Fig. 5). Specifically, isophthalaldehyde, phenol, 3-methylindole, 1-naphthalenol, phthalimide, and 3-phenyl furan were obtained. It is interesting to note the presence of nitrogen derived compounds with - amine (NH) groups such as 3-methylindole and phthalimide. It is worth mentioning that untreated wheat straw contained nitrogen element based on elemental analysis (Table 4). The presence of these nitrogenated compounds into liquid phase was justified based on the cleavage of the C-N bond due to the radical reactions. The mixture of nitrogen-compounds formed during oxidation was also mentioned elsewhere (Prado et al., 2013). In summary, solid wheat straw was undergone the degradation pathway followed by cleavage of the C-N bond to release the mixture of nitrogen-compounds in the liquid phase alongside phenolic compounds *via* H abstraction.

443

444 Fig. 5. GC/MS total ion chromatograms of phenolics obtained from photocatalysis of lignin and wheat
 445 at the optimized conditions (for lignin: formic acid concentration = 0.4 M ($x_1 = +0.33$) and TiO₂ dosage = 0.2
 446 g/L ($x_2 = +1$); for wheat straw: 0.35 M formic acid ($x_1 = +0.75$) and photocatalyst-free condition ($x_2 = -1$)).
 447 [Fixed experimental conditions: lignin loading 0.2 g/L; wheat straw loading 1 g/L; final volume 100 mL;
 448 saturated with air]. The peaks correspond to 1) guaiacol, 2) isophthalaldehyde, 3) 2-ethylphenol, 4) phenol,
 449 5) vanillin, 6) 3-methylindole, 7) 1-naphthalenol, 8) phthalimide, 9) 3-phenyl furan.

450 3.5.2. Qualification of the solid phase

451 Negligible differences ($p > 0.05$) were detected for carbon, hydrogen, and oxygen contents among untreated
 452 and treated wheat straw (Table 4). Monitoring of the nitrogen element showed that significantly ($p < 0.05$)
 453 lower content (0.46 ± 0.04 %wt) was observed in the treated sample at the optimized conditions compared to
 454 the untreated one (0.57 ± 0.00 %wt). The decrease in the nitrogen content in the wheat straw's solid fibers
 455 after CO₂^{•-} mediated reaction suggests that nitrogenated compounds in the liquid phase were produced. It
 456 also implies that H abstraction and cleavage of the C–N bond is more preferably occurring with higher rates
 457 compared to C–C bond. This result is in line with previous research when photocatalysis of nitrogenous
 458 compounds was examined (Klare et al., 2000). It is interesting to note that C/N ratio of the untreated and

459 treated samples were found to be 77.3 and 96.9, respectively. Moreover, the resulting fibers give higher
 460 heating value (HHV) of 19.33 MJ/kg (based on Gumz's formula (Channiwala and Parikh, 2002)) compared
 461 to 18.51 MJ/kg for the untreated sample, presenting a great potential to produce alternative fuels.
 462 Solid phase fibers obtained after the oxidation process of the wheat straw was characterized with XRD,
 463 FTIR, and SEM techniques and the resulting graphs are shown in Fig. 6. The XRD patterns of untreated and
 464 treated fibers are presented in Fig. 6a. The feature diffraction peaks of the treated sample exhibit similar
 465 diffraction peaks with higher intensity compared to the untreated with two prominent peaks at 18° and 22.5°
 466 corresponding to cellulose and the amorphous region, often lignin and hemicellulose, respectively (Segal et
 467 al., 1959). Crystalline cellulose associated with the intermolecular hydrogen bonding between the chains
 468 leads to the biomass recalcitrance, eliminating substrate's degradability (Lee et al., 2014). The CI expresses
 469 the ratio of crystalline cellulose to amorphous region, which is a suitable indicator to evaluate the effect of
 470 pretreatment on biomass crystallinity. A low CI indicates a high crystalline structure and a low crystalline
 471 structure is represented by a high CI (Moodley and Kana, 2017). After calculating the (CI) by Eq. (5), these
 472 values found to be 48.93% for the untreated and 51.92% for the treated, showing 3% of increment after the
 473 treatment. The increase in CI is attributed to the fact that hemicellulose and lignin in the amorphous region
 474 were partially removed during the pretreatment as mentioned above.
 475 FTIR spectra of untreated and treated wheat straw at the optimized conditions were done to investigate the
 476 change in the main functional groups and chemical structure. As shown in Fig. 6b, both samples showed a
 477 peak at 3343 cm^{-1} corresponding to O-H stretching in hydroxyl group (Kang et al., 2018). The difference in
 478 the intensity of the two samples can be attributed to the water absorption of each sample. Peaks at 2920 and
 479 2851 cm^{-1} are ascribed to C-H stretching inside cellulose which showed the higher intensity after treatment
 480 (Dehghani et al., 2015). Peak at 1732 cm^{-1} is indicative of C=O bond of hemicellulose remained the same
 481 after treatment, suggesting hemicellulose was not notably under attack during treatment (Feng Xu et al.,
 482 2013; Gandolfi et al., 2014; Mou et al., 2014). Destruction of lignin structure was shown by reduction in
 483 peak intensity at 1648 cm^{-1} (C=O linkage; conjugated carbonyls attributed to coumaryl ester group (Hage et
 484 al., 2009)) and at 1030 cm^{-1} (C-H, C-O linkages in the guaiacol structure and bond deformation (Feng Xu et
 485 al., 2013; Mainka et al., 2015; Sunthornvarabhas et al., 2017)) compared to untreated samples. Deformations

486 in aromatic rings appeared at 796 cm^{-1} , and there was no appreciable change observed among two samples
487 (Mou et al., 2014). Overall, a comparison between the untreated and treated wheat straw, lignin degradation
488 took place with various peaks belonging to lignin (1648 cm^{-1} C=O bond, 1030 cm^{-1} C-H, and C-O bonds)
489 showing a significant reduction in peaks. Moreover, peaks of hemicellulose (1732 cm^{-1} C=O conjugates) and
490 cellulose ($2920\text{-}2851\text{ cm}^{-1}$ and 1366 cm^{-1} C-H bonds) remained at largely the same shape. Hence, it can be
491 concluded that $\text{CO}_2^{\bullet-}$ assisted reaction favored removal of peaks related to lignin. TCI was calculated by
492 taking the ratio of peak areas at 1372 and 2920 cm^{-1} from FTIR spectra. TCI values were found to be 0.97
493 and 1.02 for untreated and the treated sample, respectively. The slight increase after treatment as compared
494 to untreated could be due to decrease/removal in peaks belongs to lignin, which is further corroborated the
495 results obtained by XRD analysis. Similar observation has also been obtained where an increase in CI and
496 TCI were observed (Mainka et al., 2015; Moodley and Kana, 2017; Pan et al., 2017). SEM analysis of
497 treated and untreated samples are shown in Figs. 6c and 6d. As can be seen in Fig. 6c, untreated wheat straw
498 showed long unbroken fibers with a smooth surface arranged in bundles. However, the treated samples (Fig.
499 6d) had much rougher surfaces along with cracking surface into small parts, while unbroken chains of
500 cellulose underneath were also preserved. Therefore, it can be concluded that pretreatment disrupted lignin
501 structure while preserving cellulose strands, indicating that the major biodegradable fraction has not been
502 extensively attacked. Thus, the SEM observations further strengthen XRD and FTIR results.

Fig. 6. (a) XRD and (b) FTIR spectra of the wheat straw sample before and after pretreatment, (c, d) SEM images of (c) untreated wheat straw and (d) **treated** wheat straw at the optimized conditions.

3.6. Cycle test

The cycle test was designed with the aim of re-using the formic acid solution in further pretreatment cycles. Besides the possibility of formic acid recovery, the formed phenolics are not susceptible to overoxidation. Hence, for every cycle, the content of pretreated fibers was replaced with fresh biomass using the same solution. At the end of 1st cycle, TPC increased to 65.081 ± 6.781 mg/g corresponding to $75.561 \pm 0.830\%$ increase compared to zero time (Fig. 7). The obtained result is in line with the prediction of Eq. (16) for the optimized conditions ($77.0 \pm 2.893\%$ associated with the final TPC concentration of 66.802 ± 2.281 mg/g in

the treated solution) which justifies again the validity of the model. For the 2nd and 3rd cycles the TPC increased by $44.392 \pm 3.280\%$ and $10.30 \pm 1.961\%$, respectively. The final value of TPC at the end of 2nd and 3rd cycles were 93.972 ± 3.161 mg/g and 103.651 ± 5.964 mg/g, respectively, which is comparable with the value of 94.6 mg/g reported in our previous study (Tsapekos et al., 2018). It is important to note that these observations are not in accordance with predicted results from Eq. (16). The reason for the inadequate prediction accuracy can be attributed to the declined concentration of formic acid at the beginning of 2nd and 3rd cycles. HPLC measurement was carried out to determine the concentration of formic acid at the beginning of each cycle. As shown in Fig. 7, the measurements showed a decrease in the concentration of formic acid after each cycle. At the end of 3rd cycle, the concentration of formic acid reached 0.06 ± 0.01 M.

Fig. 7. TPC and formic acid concentration during three consecutive cycles for the wheat straw sample at the optimized conditions with 0.35 M formic acid ($x_1 = +0.75$) and photocatalyst-free condition ($x_2 = -1$). [Fixed experimental conditions: wheat straw loading 1 g/L; final volume 100 mL; saturated with air].

3.7. BMP assays

Fig. 8 shows the cumulative methane production over the incubation period. 1st and 2nd cycles showed marginally equal methane production with the value of 276.7 ± 18.78 and 275.0 ± 24.32 mLCH₄/gVS, respectively. The untreated wheat straw sample reached its highest production (248.4 ± 12.29 mLCH₄/gVS) towards the end of the test whereas; the treated wheat straw sample was fully digested by the 18th day. Hence, apart from the 11.0% increase in bioenergy production, treated fibers showed also faster degradation rate, as the highest yield was produced in a shorter period compared to untreated biomass. Morales et al. (2017) conduct a BMP assay on photocatalytically treated wheat straw using TiO₂ and achieve 37% higher

methane production than untreated straw (Alvarado-Morales et al., 2017). The results of the BMP assay further validated the efficiency of the applied pretreatment process in the presence of formic acid. The lignocellulosic tissue was disrupted increasing the access for the microbes to the hemicellulose and cellulose, increasing the digestion rate and finally, aiding to reach higher methane production.

Fig. 8. BMP assay of untreated wheat straw along with 1st cycle and 2nd cycle pretreated fibers.

3.8. Implication to industrial applications

Lignin is a major discharge of 2G ethanol plant and often discarded only for its heat value due to its inherent complex structure and underdeveloped lignin valorization technologies. This waste stream is expected to exponentially increase with an increasing number of 2G bioethanol plants being built. Lignin usage is currently limited to low- and medium-value applications (e.g. binding and dispersing agents), representing a market of \$730 M (Hämäläinen et al., 2018). Other markets include vanillin production (\$192 M) and cement additives (\$176 M), as detailed by Lux Research report (Lux, 2014). To develop any strategy for lignin valorization, it would be preferred to select the pretreatment under mild conditions avoiding high pressure, temperatures, and hazardous and expensive chemical catalysts. Our proposed approach complies with this fact, thus lowering environmental impact. Apart from turning wheat straw into liquid phenolics, the process is able to generate biogas from solid leftover residue; thus complying with “zero” scheme waste production. In this regard, as far as phenolics production is concerned, 65.081 ± 6.781 and 93.972 ± 3.161 mg/g after 1st and 2nd cycle were obtained, respectively. The methane production from solid residues reached

the value of 276.7 ± 18.78 and 275.0 ± 12.29 mLCH₄/gVS after 1st and 2nd cycle. Only formic acid (0.35 M) upon a doped medium pressure UV lamp irradiation used in this method, would not influence the cost of operation significantly. The electrical energy consumption by the UV-lamp was considered as solely energy used during the pretreatment. For the used lamp, a value of 0.061 kWh per unit of volume (m³) of the suspension treated per unit of time (min) was determined experimentally according to Hansen et al. (Hansen et al., 2013). Hence, HCOOH reaction upon a doped medium pressure UV lamp irradiation is believed to push forward the future prospects of biorefineries with greener pretreatment process and use more efficiently feedstocks and wastes in a circular economy-frame.

4. Conclusion

A novel UV/TiO₂/HCOOH reaction upon a doped medium pressure UV lamp irradiation was proposed to depolymerize lignin for production phenolic compounds. Efficient H abstraction from 2,3-dihydrobenzofuran as β-O-4 linkages representative of lignin was observed. The addition of methyl viologen proved that CO₂^{•-} was mainly responsible for TPC production. This pretreatment method was adopted to test the technical feasibility for production of high value products from lignin and wheat straw as an actual lignocellulosic biomass. The highest concertation of TPC obtained from lignin was 173.431 ± 2.970 mg/g at optimized conditions of 0.4 M formic acid and 0.2 g/L TiO₂. The highest TPC achieved in the case of wheat straw was 66.802 ± 2.281 mg/g corresponded to the optimal point of 0.35 M formic acid and free-photocatalyst condition. It was found that free-photocatalyst condition promoted the production of TPC in the presence of formic acid. The highest TPC production after 3rd cycle was 103.651 ± 5.964 mg/g, highlighting the advantage of this pretreatment for producing valuable compounds under facile environment. The solid leftover products showed increased methane production, achieving 11.0% increase compared to the untreated wheat straw. This work provides an environment-friendly pretreatment alternative for effective production of high-value products (phenolics and methane).

Acknowledgments

The authors thank Hector Garcia for technical assistance.

References

579 Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates
580 in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* 2, 875–877. doi:10.1038/nprot.2007.102

581 Alvarado-Morales, M., Tsapekos, P., Awais, M., Gulfraz, M., Angelidaki, I., 2017. TiO₂ /UV based
582 photocatalytic pretreatment of wheat straw for biogas production. *Anaerobe* 46, 155–161.
583 doi:10.1016/j.anaerobe.2016.11.002

584 Angelidaki, I., Karakashev, D., Batstone, D.J., Plugge, C.M., Stams, A.J.M., 2011. Biomethanation and Its
585 Potential. *Methods Methane Metab. Part A* 494, 327–351. doi:10.1016/B978-0-12-385112-3.00016-0

586 APHA, 1998. *Standard Methods for Examination of Water and Wastewater*, American Public Health
587 Association (APHA). Washington, DC, USA.

588 Berkovic, A.M., Bertolotti, S.G., Villata, L.S., Gonzalez, M.C., Pis Diez, R., Mártire, D.O., 2012.
589 Photoinduced reduction of divalent mercury by quinones in the presence of formic acid under anaerobic
590 conditions. *Chemosphere* 89, 1189–1194. doi:10.1016/J.CHEMOSPHERE.2012.07.019

591 Berkovic, A.M., Gonzalez, M.C., Russo, N., del Carmen Michelini, M., Diez, R.P., Mártire, D.O., 2010.
592 Reduction of Mercury(II) by the Carbon Dioxide Radical Anion: A Theoretical and Experimental
593 Investigation. *J. Phys. Chem. A* 114, 12845–12850. doi:10.1021/jp106035m

594 Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K.B., Ramakrishnan, S., 2011. Chemical and
595 Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. *Enzyme Res.* 2011, 1–17.
596 doi:10.4061/2011/787532

597 Channiwala, S.A., Parikh, P.P., 2002. A unified correlation for estimating HHV of solid, liquid and gaseous
598 fuels. *Fuel* 81, 1051–1063. doi:10.1016/S0016-2361(01)00131-4

599 Chen, J., Liu, W., Song, Z., Wang, H., Xie, Y., 2018. Photocatalytic Degradation of β -O-4 Lignin Model
600 Compound by In₂S₃ Nanoparticles Under Visible Light Irradiation. *Bioenergy Res.* 11, 166–173.
601 doi:10.1007/s12155-017-9886-8

602 Dehghani, M., Karimi, K., Sadeghi, M., 2015. Pretreatment of rice straw for the improvement of biogas

603 production. *Energy and Fuels* 29, 3770–3775. doi:10.1021/acs.energyfuels.5b00718

604 Dong, H., Zeng, G., Tang, L., Fan, C., Zhang, C., He, X., He, Y., 2015. An overview on limitations of TiO₂-
605 based particles for photocatalytic degradation of organic pollutants and the corresponding
606 countermeasures. *Water Res.* 79, 128–146. doi:10.1016/j.watres.2015.04.038

607 Feng, P., Wang, H., Lin, H., Zheng, Y., 2019. Selective production of guaiacol from black liquor: Effect of
608 solvents. *Carbon Resour. Convers.* 2, 1–12. doi:10.1016/J.CRCON.2018.07.005

609 Feng Xu, Jianming Yu, Tesfaye Tesso, Floyd Dowell, Donghai Wang, 2013. Qualitative and quantitative
610 analysis of lignocellulosic biomass using infrared techniques : A mini-review. *Appl. Energy* 104, 801–
611 809. doi:10.1016/j.apenergy.2012.12.019

612 Gandolfi, S., Ottolina, G., Consonni, R., Riva, S., Patel, I., 2014. Fractionation of hemp hurds by organosolv
613 pretreatment and its effect on production of lignin and sugars. *ChemSusChem* 7, 1991–1999.
614 doi:10.1002/cssc.201301396

615 Gong, J., Imbault, A., Farnood, R., 2017. The promoting role of bismuth for the enhanced photocatalytic
616 oxidation of lignin on Pt-TiO₂ under solar light illumination. *Appl. Catal. B Environ.* 204, 296–303.
617 doi:10.1016/j.apcatb.2016.11.045

618 Gu, X., Lu, S., Fu, X., Qiu, Z., Sui, Q., Guo, X., 2017. Carbon dioxide radical anion-based
619 UV/S₂O₈²⁻/HCOOH reductive process for carbon tetrachloride degradation in aqueous solution. *Sep.*
620 *Purif. Technol.* 172, 211–216. doi:10.1016/J.SEPPUR.2016.08.019

621 Hage, R. El, Brosse, N., Chrusciel, L., Sanchez, C., Sannigrahi, P., Ragauskas, A., 2009. Characterization of
622 milled wood lignin and ethanol organosolv lignin from miscanthus. *Polym. Degrad. Stab.* 94, 1632–
623 1638. doi:10.1016/j.polymdegradstab.2009.07.007

624 Hämäläinen, V., Grönroos, T., Suonpää, A., Heikkilä, M.W., Romein, B., Ihalainen, P., Malandra, S., Birikh,
625 K.R., 2018. Enzymatic Processes to Unlock the Lignin Value . *Front. Bioeng. Biotechnol.* .

626 Hansen, K.M.S., Zortea, R., Piketty, A., Rodriguez, S., Rasmus, H., 2013. Photolytic removal of DBPs by

627 medium pressure UV in swimming pool water. *Sci. Total Environ.* 443, 850–856.
 628 doi:10.1016/j.scitotenv.2012.11.064

629 Kang, X., Sun, Y., Li, L., Kong, X., Yuan, Z., 2018. Improving methane production from anaerobic
 630 digestion of Pennisetum Hybrid by alkaline pretreatment. *Bioresour. Technol.* 255, 205–212.
 631 doi:10.1016/j.biortech.2017.12.001

632 Kansal, S.K., Singh, M., Sud, D., 2008. Studies on TiO₂/ZnO photocatalysed degradation of lignin. *J.*
 633 *Hazard. Mater.* 153, 412–417. doi:10.1016/j.jhazmat.2007.08.091

634 Kärkäs, M.D., Matsuura, B.S., Monos, T.M., Magallanes, G., Stephenson, C.R.J., 2016. Transition-metal
 635 catalyzed valorization of lignin: the key to a sustainable carbon-neutral future. *Org. Biomol. Chem.* 14,
 636 1853–1914. doi:10.1039/C5OB02212F

637 Khoshnevisan, B., Tsapekos, P., Alvarado-Morales, M., Angelidaki, I., 2018. Process performance and
 638 modelling of anaerobic digestion using source-sorted organic household waste. *Bioresour. Technol.*
 639 247, 486–495. doi:10.1016/j.biortech.2017.09.122

640 Klare, M., Scheen, J., Vogelsang, K., Jacobs, H., Broekaert, J.A.C., 2000. Degradation of short-chain alkyl-
 641 and alkanolamines by TiO₂- and Pt/TiO₂-assisted photocatalysis. *Chemosphere* 41, 353–362.
 642 doi:10.1016/S0045-6535(99)00447-6

643 Lee, H. V., Hamid, S.B.A., Zain, S.K., 2014. Conversion of Lignocellulosic Biomass to Nanocellulose:
 644 Structure and Chemical Process. *Sci. World J.* 2014, 1–20. doi:10.1155/2014/631013

645 Li, H., Lei, Z., Liu, C., Zhang, Z., Lu, B., 2015. Photocatalytic degradation of lignin on synthesized Ag–
 646 AgCl/ZnO nanorods under solar light and preliminary trials for methane fermentation. *Bioresour.*
 647 *Technol.* 175, 494–501. doi:10.1016/j.biortech.2014.10.143

648 Lux, 2014. Finding untapped value: converting lignin to higher value chemicals. Available at
 649 <https://members.luxresearchinc.com/research/report/14807>.

650 Ma, Y.-S., Chang, C.-N., Chiang, Y.-P., Sung, H.-F., Chao, A.C., 2008. Photocatalytic degradation of lignin

651 using Pt/TiO₂ as the catalyst. *Chemosphere* 71, 998–1004. doi:10.1016/j.chemosphere.2007.10.061

652 Machado, A.E.H., Furuyama, A.M., Falone, S.Z., Ruggiero, R., Perez, D.D.S., Castellan, A., 2000.

653 Photocatalytic degradation of lignin and lignin models, using titanium dioxide: the role of the hydroxyl

654 radical. *Chemosphere* 40, 115–124. doi:10.1016/S0045-6535(99)00269-6

655 Mainka, H., Hilfert, L., Busse, S., Edelmann, F., Haak, E., Herrmann, A.S., 2015. Characterization of the

656 major reactions during conversion of lignin to carbon fiber. *J. Mater. Res. Technol.* 4, 377–391.

657 doi:10.1016/j.jmrt.2015.04.005

658 Menon, V., Rao, M., 2012. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals &

659 biorefinery concept. *Prog. Energy Combust. Sci.* doi:10.1016/j.pecs.2012.02.002

660 Moodley, P., Kana, E.B.G., 2017. Microwave-assisted inorganic salt pretreatment of sugarcane leaf waste:

661 Effect on physiochemical structure and enzymatic saccharification. *Bioresour. Technol.* 235, 35–42.

662 doi:10.1016/j.biortech.2017.03.031

663 Mou, H., Li, B., Fardim, P., 2014. Pretreatment of corn stover with the modified hydrotropic method to

664 enhance enzymatic hydrolysis. *Energy and Fuels* 28, 4288–4293. doi:10.1021/ef5001634

665 Nair, V., Dhar, P., Vinu, R., 2016. Production of phenolics via photocatalysis of ball milled lignin-

666 TiO₂ mixtures in aqueous suspension. *RSC Adv.* 6, 18204–18216. doi:10.1039/c5ra25954a

667 Nelson, M.L., O'Connor, R.T., 1964. Relation of certain infrared bands to cellulose crystallinity and crystal

668 lattice type. Part II. A new infrared ratio for estimation of crystallinity in celluloses I and II. *J. Appl.*

669 *Polym. Sci.* 8, 1325–1341. doi:10.1002/app.1964.070080323

670 Pan, M., Gan, X., Mei, C., Liang, Y., 2017. Structural analysis and transformation of biosilica during

671 lignocellulose fractionation of rice straw. *J. Mol. Struct.* 1127, 575–582.

672 doi:10.1016/j.molstruc.2016.08.002

673 Pérez, J., Muñoz-Dorado, J., de la Rubia, T., Martínez, J., 2002. Biodegradation and biological treatments of

674 cellulose, hemicellulose and lignin: an overview. *Int. Microbiol.* 5, 53–63. doi:10.1007/s10123-002-

675 0062-3

676 Pineda, A., Lee, A.F., 2016. Heterogeneously catalyzed lignin depolymerization. *Appl. Petrochemical Res.*
677 6, 243–256. doi:10.1007/s13203-016-0157-y

678 Prado, R., Erdocia, X., Labidi, J., 2013. Effect of the photocatalytic activity of TiO₂ on lignin
679 depolymerization. *Chemosphere* 91, 1355–1361. doi:10.1016/j.chemosphere.2013.02.008

680 Rauf, M.A., Ashraf, S.S., 2009. Fundamental principles and application of heterogeneous photocatalytic
681 degradation of dyes in solution. *Chem. Eng. J.* 151, 10–18. doi:10.1016/j.cej.2009.02.026

682 Ren, H., Hou, Z., Han, X., Zhou, R., 2017. Highly reductive radical CO₂ – deriving from a system with SO
683 4 – and formate anion: Implication for reduction of Cr(VI) from wastewater. *Chem. Eng. J.* 309, 638–
684 645. doi:10.1016/j.cej.2016.10.071

685 Rosso, J.A., Bertolotti, S.G., Braun, A.M., Mártire, D.O., Gonzalez, M.C., 2001. Reactions of carbon dioxide
686 radical anion with substituted benzenes. *J. Phys. Org. Chem.* 14, 300–309. doi:10.1002/poc.365

687 Segal, L., Creely, J.J., Martin Jr, A.E., Conrad, C.M., 1959. An empirical method for estimating the degree
688 of crystallinity of native cellulose using the X-ray diffractometer. *Text. Res. J.* 29, 786–794.

689 Smolarski, N., 2012. High-value opportunities for lignin: unlocking its potential. *Frost & Sullivan* 1.

690 Sun, Z., Fridrich, B., de Santi, A., Elangovan, S., Barta, K., 2018. Bright Side of Lignin Depolymerization:
691 Toward New Platform Chemicals. *Chem. Rev.* 118, 614–678. doi:10.1021/acs.chemrev.7b00588

692 Sunthornvarabhas, J., Liengprayoon, S., Suwonsichon, T., 2017. Antimicrobial kinetic activities of lignin
693 from sugarcane bagasse for textile product. *Ind. Crops Prod.* 109, 857–861.
694 doi:10.1016/j.indcrop.2017.09.059

695 Todres, Z.V., 2009. Ion-radical organic chemistry: principles and applications, *Chemistry & biodiversity.*
696 CRC Press.

697 Tsapekos, P., Alvarado-Morales, M., Boscaro, D., Mazarji, M., Sartori, L., Angelidaki, I., 2018. TiO₂ –

698 AgCl Based Nanoparticles for Photocatalytic Production of Phenolic Compounds from Lignocellulosic
699 Residues. *Energy & Fuels* 32, 6813–6822. doi:10.1021/acs.energyfuels.8b00572

700 Tsapekos, P., Kougias, P.G., Vasileiou, S.A., Lyberatos, G., Angelidaki, I., 2017. Effect of micro-aeration
701 and inoculum type on the biodegradation of lignocellulosic substrate. *Bioresour. Technol.* 225, 246–
702 253. doi:10.1016/j.biortech.2016.11.081

703 Wang, M., Ma, J., Liu, H., Luo, N., Zhao, Z., Wang, F., 2018. Sustainable Productions of Organic Acids and
704 Their Derivatives from Biomass via Selective Oxidative Cleavage of C–C Bond. *ACS Catal.* 8, 2129–
705 2165. doi:10.1021/acscatal.7b03790

706 Zhang, J., Cai, Y., Lu, G., Cai, C., 2016. Facile and selective hydrogenolysis of β -O-4 linkages in lignin
707 catalyzed by Pd–Ni bimetallic nanoparticles supported on ZrO₂. *Green Chem.* 18, 6229–6235.
708 doi:10.1039/C6GC02265K

709 Zoschke, K., Börnick, H., Worch, E., 2014. Vacuum-UV radiation at 185 nm in water treatment – A review.
710 *Water Res.* 52, 131–145. doi:10.1016/j.watres.2013.12.034

711

712 TABLES

713 Table 1. Experimental ranges and levels of independent variables.

Variables	Coded variables (x_i)	Unit	Sample names	Ranges and levels		
				-1	0	+1
Formic acid	x_1	M	Lignin	0	0.3	0.6
			Wheat straw	0	0.2	0.4
TiO ₂	x_2	g/L	Lignin	0	0.1	0.2
			Wheat straw	0	0.05	0.1

714

715 Table 2. fCCD design matrix for two variables with observed values for lignin and wheat straw samples.

Run order	Coded values		Lignin	Wheat Straw	
	x_1	x_2	Exp. TPC (%)	Exp. TPC (%)	Exp. sCOD (%)
1	+1	-1	11.68 ± 2.70	76.33 ± 1.69	14.56 ± 6.32
2	+1	0	33.30 ± 2.53	66.47 ± 0.19	25.85 ± 5.89
3	+1	+1	48.56 ± 6.95	62.91 ± 3.00	19.66 ± 5.41
4	0	-1	41.41 ± 2.66	68.94 ± 2.57	34.88 ± 15.04
5	0	+1	59.51 ± 2.79	70.92 ± 0.52	32.00 ± 10.18
6	0	0	53.56 ± 0.93	72.12 ± 4.59	63.00 ± 5.17

7	0	0	53.00 ± 0.91	70.00 ± 3.67	60.00 ± 4.89
8	-1	+1	-23.90 ± 1.84	19.89 ± 3.06	97.08 ± 8.11
9	-1	0	-6.16 ± 1.85	21.07 ± 0.66	100.00 ± 9.98
10	-1	-1	3.18 ± 0.80	13.92 ± 1.49	95.00 ± 12.84

716

717 Table 3. Major products obtained at the optimized conditions for in the solution phase of lignin and wheat
718 straw sample by GC/MS.

Peak No.	Identified compounds	Retention time (min)	Molecular weight (g/mol)	m/z
1	Guaiacol	12.80 min	124.14	124, 109, 95, 81, 65, 53
2	Isophthalaldehyde	12.86 min	134.13	134, 105, 77, 51
3	2-ethylphenol	14.95 min	122.17	122, 107, 91, 77, 51
4	Phenol	15.03 min	94	94, 66, 55
5	Vanillin	19.30 min	152.15	151, 137, 109, 81, 53
6	3-methylindole	19.55 min	131.17	130, 103, 89, 77, 65
7	1-naphthalenol	22.88 min	144.17	144, 115, 89, 72, 63
8	Phthalimide	22.96 min	147.13	147, 119, 104, 90, 76, 50
9	3-phenyl furan	23.39 min	144.17	144, 115, 89, 72, 63

719

720 Table 4. Elemental analysis of untreated and treated solid wheat straw's fiber at the optimized conditions.

samples	Elemental content (wt %)			
	C	H	N	O
Untreated wheat straw	44.05 ± 0.54	6.66 ± 4.24	0.57 ± 0.00	48.72 ± 3.55
Treated wheat straw	44.91 ± 0.30	7.00 ± 0.67	0.46 ± 0.04	47.63 ± 0.07

721

723

724 Fig. 1. Mole fraction profiles of 2,3-dihydrobenzofuran oxidation and its obtained products as a function of
 725 time. Conditions: 2,3-dihydrobenzofuran loading 0.2 g/L; formic acid concentration 0.25 M; TiO₂ dosage 0.1
 726 g/ L; final volume 100 mL; saturated with air.

727

728 Fig. 2. TPC concentration (mg/g) as a function of time with the addition of different methyl viologen (MV²⁺)
 729 concentration at center point (experiments 6–7). Fixed experimental conditions: lignin loading 0.2 g/L; final
 730 volume 100 mL; saturated with air.

Fig. 3. TPC concentration (mg/g) as a function of time under different conditions according to the fCCD for a) lignin and b) wheat straw. Center point corresponds to the average of experiments 6–7. Fixed experimental conditions: lignin loading 0.2 g/L; wheat straw loading 1 g/L; final volume 100 mL; saturated with air.

Fig. 4. Surface plots of a) TPC % change as a function of formic acid concentration (x_1) and TiO_2 dosage (x_2) at irradiation time of 180 min for lignin, and surface plots of b) TPC % change, and c) sCOD % change as a function of formic acid concentration (x_1) and TiO_2 dosage (x_2) at irradiation time of 240 min for wheat straw. [Fixed experimental conditions: lignin loading 0.2 g/L; wheat straw loading 1 g/L; final volume 100 mL; saturated with air].

741

742

743 Fig. 5. GC/MS total ion chromatograms of phenolics obtained from photocatalysis of lignin and wheat
 744 at the optimized conditions (for lignin: formic acid concentration = 0.4 M ($x_1 = +0.33$) and TiO_2 dosage = 0.2
 745 g/L ($x_2 = +1$); for wheat straw: 0.35 M formic acid ($x_1 = +0.75$) and photocatalyst-free condition ($x_2 = -1$)).
 746 [Fixed experimental conditions: lignin loading 0.2 g/L; wheat straw loading 1 g/L; final volume 100 mL;
 747 saturated with air]. The peaks correspond to 1) guaiacol, 2) isophthalaldehyde, 3) 2-ethylphenol, 4) phenol,
 748 5) vanillin, 6) 3-methylindole, 7) 1-naphthalenol, 8) phthalimide, 9) 3-phenyl furan.

749

750 Fig. 6. (a) XRD and (b) FTIR spectra of wheat straw sample before and after pretreatment, (c, d) SEM

751 images of (c) untreated wheat straw and (d) **treated** wheat straw at the optimized conditions.

752

753

754 Fig. 7. TPC and formic acid concentration during three consecutive cycles for wheat straw sample at the
 755 optimized conditions with 0.35 M formic acid ($x_1 = +0.75$) and photocatalyst-free condition ($x_2 = -1$). [Fixed
 756 experimental conditions: wheat straw loading 1 g/L; final volume 100 mL; saturated with air].

757

758 Fig. 8. BMP assay of untreated wheat straw along with 1st cycle and 2nd cycle pretreated fibers.