# Supplementary Information

## Integrated Biorefinery Routes to Transform Furfural Waste into 2G Biofuels and PFOA-Adsorbing Biochar

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## 1. Supplementary Methods

### *1.1. Physicochemical characterization of raw and pretreated FRs*

Fourier transform infrared spectroscopy (FTIR, Thermo Scientific, USA) was employed to analyze the chemical functional groups in both raw and pretreated FRs. The spectra were recorded over a wavenumber range of 500 to 4000 $cm^{-1}$, using 32 scans at a resolution of 4 $cm^{-1}$.[1] To assess the crystallinity index (CrI) of cellulose in the raw and pretreated FRs, X-ray diffraction (XRD, Bruker D8) was utilized. Measurements were taken across a diffraction angle (2θ) range of 5º to 50º, with a scan rate of 5º·$min^{-1}$. The CrI was calculated according to Eq. 1.[2]

$$\text{CrI}\ (\%) = \frac{I_{max} - I_{min}}{I_{max}} \times 100 \qquad (1)$$

where the maximum and minimum peaks of the 2$\theta$ are at 22.5° and 18.5°, respectively.

The specific surface area, average pore volume, and average pore diameter of the FRs were determined using a surface area analyzer (Tristar 3020 II, USA) *via* the Brunauer-Emmett-Teller (BET) method, which facilitated the evaluation of sample porosity. Nitrogen adsorption was conducted at -196 °C, followed by desorption at 60 °C for 12 h.[3] Additionally, scanning electron microscopy (SEM, Hitachi Regulus8100) was employed to investigate the surface morphology, structural characteristics, and micro-morphological changes of the FRs.[4]

### *1.2. Lignin extraction*

Take 10 g of the pretreated material and place it in a 250 mL Erlenmeyer flask for enzymatic hydrolysis. Add 100 mL of citrate buffer (pH 4.8, containing 20 FPU·$g^{-1}$ Cellic® CTec 3 enzyme preparation) and mix thoroughly. Incubate at 50°C for 48 h. After enzymatic

hydrolysis, centrifuge the mixture at 8000 rpm for 5 min to separate the supernatant, retaining the residue. Wash the residue with hot water to remove soluble sugars and cellulase enzymes. The washed residue is then subjected to freeze-drying.

The freeze-dried solid sample is extracted with 96% dioxane under light protection (solid-to-liquid ratio of 1:20) and stirred at room temperature for 24 h. This extraction is repeated twice. Following extraction, centrifuge to remove any solid phase, then evaporate the supernatant at 45°C to concentrate it to approximately 40 mL. Subsequently, the concentrated solution is dipped 10 times its volume of hydrochloric acid solution (pH = 2.0), precipitating the lignin, which is then collected. Finally, the precipitate is freeze-dried to obtain the lignin sample.

*1.3 Pyrolysis process*

Following an established protocol, the pyrolysis process was conducted at the New Energy and Environmental Laboratory (NEEL) at Chengdu University.[5] Both raw FRs (as control) and remaining residues after cellulose hydrolysis were subjected to analysis in a fixed-bed horizontal quartz reactor, measuring 500 mm in length and 50 mm in internal diameter. In each experiment, 5 g of the sample was placed in a ceramic crucible and introduced into the reactor. Nitrogen was introduced at a flow rate of 65 $mL \cdot min^{-1}$ to create an inert atmosphere. The temperature was gradually increased to 550°C over a 15-min period, informed by thermogravimetric (TG) analysis, and maintained at this maximum for 20 min to ensure complete carbonization of lignin while avoiding unnecessary energy waste. The reactor's condensation system consisted of three sequential collection tanks maintained at temperatures of 80 °C, 25 °C, and 4 °C to capture the condensable vapors effectively. Non-condensable gases

were expelled through an exhaust outlet. Upon completion of the pyrolysis, the reactor was allowed to cool to room temperature, after which the biochar was collected and weighed. Changes in the reactor's mass, condensation units, and associated connections before and after pyrolysis were utilized to calculate the yields of the various products. The yields of bio-oil ($Y_O$), biochar ($Y_b$), and gas ($Y_g$) were then determined using Eq. 6–8;

$$Y_O\,(\%) = \frac{A_a - A_b}{M_T} \times 100 \qquad (6)$$

$$Y_b\,(\%) = \frac{B_b - B_a}{V_t} \times 100 \qquad (7)$$

$$Y_g\,(\%) = 100 - (Y_O + Y_b) \qquad (8)$$

where $A_a$ and $A_b$ refer to the masses of the jars, connections, and reactor chamber after and before the pyrolysis, respectively. $B_b$ and $B_a$ indicate the masses of the sample vessel with substrate before and after pyrolysis, respectively, while $V_t$ represents the initial mass of the substrate.

*1.4. Physicochemical characterization of biochar samples*

The biochar samples, both pre- and post-PFOA adsorption, were subjected to comprehensive characterization through pore structure analysis and SEM as outlined in section 2.3. Additionally, X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha, USA) was utilized to investigate the surface elemental composition and to identify predominant functional groups.[6] Spectral deconvolution was performed using the Thermo Advantage software. Surface wettability was evaluated by measuring the contact angle of biochar samples with a goniometer (SDC 350KS, China). The pH of the biochar samples was measured

following a modified procedure by Ndoun.[7] Biochar was added to deionized water at a mass ratio of 1:20 (0.5 g biochar to 10 mL deionized water). The mixture was shaken using a mechanical shaker for 1 hour, and the pH was then measured.

*1.5. bio-oil characterization*

After the pyrolysis experiment, the resulting bio-oil samples underwent filtration through a 0.22 μm microporous membrane before gas chromatography-mass spectrometry (GC-MS) analysis. For this purpose, a 7890A-5975C gas chromatograph (Agilent Technologies, Inc., USA) equipped with an HP-5MS column (30.0 m × 250 μm, 0.25 μm) was employed. The analysis started at an initial temperature of 60 °C, held for 1 min, and then increased to 200 °C at a rate of 10 °C·min$^{-1}$. The temperature was subsequently raised to 300 °C at 5 °C·min$^{-1}$, with a 5-min hold. The injector was set at 280 °C, and the transfer line at 300 °C, with helium serving as the carrier gas at a flow rate of 1.0 mL·min$^{-1}$. The split ratio was adjusted to 50:1, and a 1 μL injection volume was used.

First, the compounds in the GC-MS data are categorized into groups (such as Phenolic compounds, sugar, acids, esters, hydrocarbons, etc.), and the sum of the percentage for each category is calculated. Then, the percentage of each category is multiplied by its corresponding percentage in the bio-oil to determine the relative percentages of that category in the bio-oil.

## 2. Supplementary Tables

**Table S1** Effect of acid/glycerol pretreatment on chemical compositions of FRs at different types of surfactants and temperatures. Surfactant dosage: 5%

| Temperature | Pretreatment | Solid recovery (%) | Component distribution | | | | Cellulose recovery (%) | Delignification (%) |
|---|---|---|---|---|---|---|---|---|
| | | | Cellulose (%) | Xylan (%) | Lignin (%) | Ash (%) | | |
| | Raw material | | 48.9 ± 0.2 | 2.1 ± 0.4 | 47.6 ± 0.6 | 1.4 ± 0.3 | | |
| 100 ℃ | Control | 93.8 ± 0.1 | 48.6 ± 0.2 | 1.3 ± 0.3 | 48.7 ± 0.1 | 0.4 ± 0.2 | 93.2 ± 0.3 | 4.2 ± 0.2 |
| | + PEG 4000 | 97.1 ± 0.1 | 47.6 ± 0.1 | 1.2 ± 0.3 | 47.6 ± 0.2 | 0.3 ± 0.2 | 94.5 ± 0.2 | 3.0 ± 0.1 |
| | + Tween 80 | 98.0 ± 0.3 | 47.1 ± 0.2 | 1.1 ± 0.6 | 48.2 ± 0.2 | 0.4 ± 0.1 | 94.4 ± 0.1 | 0.8± 0.1 |
| | + Triton-X100 | 96.7 ± 0.3 | 47.4 ± 0.3 | 1.2 ± 0.4 | 48.1 ± 0.3 | 0.4 ± 0.1 | 93.7 ± 0.1 | 2.4 ± 0.3 |
| | + AEO | 97.9 ± 0.2 | 48.9 ± 0.3 | 1.3 ± 0.2 | 48.4 ± 0.3 | 0.4 ± 0.1 | 98.0 ± 0.3 | 0.6 ± 0.2 |
| **120 ℃** | Control | 94.4 ± 0.4 | 45.9 ± 0.4 | 1.2 ± 0.1 | 48.6 ± 0.3 | 0.6 ± 0.1 | 88.5 ± 0.3 | 3.8± 0.2 |
| | + PEG 4000 | 96.2 ± 0.3 | 46.4 ± 0.5 | 1.2 ± 0.2 | 48.0 ± 0.5 | 0.5 ± 0.3 | 91.2 ± 0.4 | 3.0 ± 0.1 |
| | + Tween 80 | 95.7 ± 0.6 | 47.6 ± 0.1 | 1.2 ± 0.3 | 49.6 ± 0.3 | 0.4 ± 0.2 | 93.1 ± 0.2 | 4.2 ± 0.2 |
| | + Triton-X100 | 94.2 ± 0.3 | 46.5 ± 0.3 | 1.2 ± 0.2 | 48.2 ± 0.4 | 0.6 ± 0.2 | 89.6 ± 0.6 | 4.6 ± 0.3 |
| | **+ AEO** | **97.4 ± 0.3** | **48.6 ± 0.2** | **1.2 ± 0.1** | **48.2 ± 0.3** | **0.5 ± 0.3** | **96.6 ± 0.3** | **1.6 ± 0.1** |
| 140 ℃ | Control | 88.7 ± 0.2 | 43.9 ± 0.2 | 1.1 ± 0.1 | 48.7 ± 0.2 | 0.4 ± 0.1 | 79.7 ± 0.2 | 9.2 ± 0.8 |
| | + PEG 4000 | 90.7 ± 0.3 | 45.8 ± 0.4 | 1.1 ± 0.2 | 48.7 ± 0.2 | 0.6 ± 0.2 | 84.8 ± 0.2 | 7.4 ± 0.4 |
| | + Tween 80 | 90.2 ± 0.6 | 43.8 ± 0.7 | 1.2 ± 0.2 | 49.2 ± 0.2 | 0.5 ± 0.1 | 80.8 ± 0.1 | 6.8 ± 0.3 |
| | + Triton-X100 | 89.2 ± 0.1 | 44.4 ± 0.1 | 1.1 ± 0.1 | 50.3 ± 0.3 | 0.35 ± 0.1 | 81.0 ± 0.4 | 6.0 ± 0.2 |
| | + AEO | 90.1 ± 0.2 | 45.3 ± 0.1 | 1.1 ± 0.0 | 49.7 ± 0.7 | 0.38 ± 0.2 | 83.5 ± 0.4 | 6.0 ± 0.1 |
| 160 ℃ | Control | 81.4 ± 0.3 | 40.8 ± 0.4 | 0.7 ± 0.1 | 54.3 ± 0.5 | 0.7 ± 0.2 | 67.9 ± 0.2 | 7.2 ± 0.4 |
| | + PEG 4000 | 85.1 ± 0.6 | 39.2 ± 0.6 | 1.0 ± 0.3 | 53.2 ± 0.3 | 0.4 ± 0.2 | 68.1± 0.3 | 5.0 ± 0.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | + Tween 80 | 83.2 ± 0.5 | 41.6 ± 0.3 | 1.1 ± 0.3 | 53.9 ± 0.7 | 0.8 ± 0.1 | 70.6 ± 0.5 | 6.0 ± 0.1 |
| | + Triton-X100 | 84.5 ± 0.3 | 41.3 ± 0.3 | 0.9 ± 0.2 | 54.1 ± 1.0 | 0.7 ± 0.2 | 71.3 ± 0.1 | 4.1 ± 0.4 |
| | + AEO | 86.0 ± 0.3 | 40.2 ± 0.2 | 0.7 ± 0.4 | 53.7 ± 0.6 | 0.4 ± 0.2 | 70.6 ± 0.3 | 3.2 ± 0.2 |

**Table S2** Effect of different dosages of AEO in acid/glycerol pretreatment on the distribution of chemical compositions and cellulose hydrolysis.

| AEO dosage (%) | Solid recovery (%) | Component distribution | | | | Cellulose recovery (%) | Delignification (%) | Cellulose hydrolysis (%), 48 h |
|---|---|---|---|---|---|---|---|---|
| | | Cellulose (%) | Xylan (%) | Lignin (%) | Ash (%) | | | |
| 0 | 94.4 ± 0.4 | 45.9 ± 0.4 | 1.2 ± 0.1 | 48.6 ± 0.3 | 0.6 ± 0.1 | 88.5 ± 0.3 | 3.8 ± 0.2 | 54.1 ± 0.2 |
| 1 | 96.3 ± 0.3 | 47.9 ± 0.2 | 1.5 ± 0.1 | 48.1 ± 0.3 | 1.0 ± 0.3 | 94.3 ± 0.3 | 2.7 ± 0.2 | 72.2 ± 0.7 |
| 3 | 97.0 ± 1.2 | 48.1 ± 0.5 | 1.4 ± 0.3 | 48.2 ± 0.2 | 1.2 ± 0.4 | 95.4 ± 0.2 | 1.8 ± 0.3 | 77.1 ± 0.5 |
| **5** | **97.4 ± 0.3** | **48.6± 0.2** | **1.2 ± 0.1** | **48.2 ± 0.4** | **0.5 ± 0.1** | **96.6 ± 0.4** | **1.6± 0.1** | **85.5 ± 0.7** |
| **7** | 95.5 ± 0.3 | 48.7 ± 0.1 | 1.5 ± 0.2 | 48.7 ± 0.4 | 1.1 ± 0.3 | 95.1 ± 0.4 | 2.4 ± 0.1 | 84.1 ± 0.3 |

**Table S3** Ethanol yield and productivity obtained from SHF of pretreated furfural residues.

| Pretreatment | Biomass loading（%，w/v） | Max glucose in hydrolysis（$g \cdot L^{-1}$） | Max glucose in hydrolysis（%） | Max ethanol（$g \cdot L^{-1}$） | $Y_{ethanol}/g$（$g \cdot g^{-1}$） | Theoretical max bioethanol yield（%） |
|---|---|---|---|---|---|---|
| **Acid/glycerol** | 20 | 52.6 ± 1.4 | 51.6 ± 0.7 | 22.0 ± 0.4 | 0.12 | 91.1 |
| **Acid/glycerol + AEO** | 20 | 87.5 ± 0.8 | 82.5 ± 0.4 | 36.7 ± 0.6 | 0.20 | 91.4 |

## References


1. M. Madadi, M. Elsayed, G. Song, R. Kumar, M. Mahmoud-Aly, B. Basak, B.-H. Jeon and F. Sun, *Chemical Engineering Journal*, 2023, **465**, 142881.
2. B. J. Ma, Y. Sun, K. Y. Lin, B. Li and W. Y. Liu, *Bioresource technology*, 2014, **156**, 189-194.
3. P. Sinha, A. Datar, C. Jeong, X. Deng, Y. G. Chung and L.-C. Lin, *The Journal of Physical Chemistry C*, 2019, **123**, 20195-20209.
4. M. Elsayed, M. Madadi, G. Song, Z. Zhou, H. Wang, J. Wang, J. Zhang, M. Aghbashlo and M. Tabatabaei, *Fuel*, 2024, **376**, 132678.
5. M. Xiong, J. Huang, X. He, Z. Zhou, X. Qu, S. Faisal and A. Abomohra, *Fuel*, 2023, **331**, 125710.
6. Z.-Z. Liu, C.-G. Pan, F.-J. Peng, J.-J. Hu, H.-M. Tan, R.-G. Zhu, C.-Y. Zhou, H. Liang and K. Yu, *Bioresource Technology*, 2024, **408**, 131157.
7. M. C. Ndoun, H. A. Elliott, H. E. Preisendanz, C. F. Williams, A. Knopf and J. E. Watson, *Biochar*, 2021, **3**, 89-104.