

Improved reductive catalytic fractionation of lignocellulosic biomass through the application of a recyclable magnetic catalyst

Electronic Supporting Information

Federico Bugli,^{a,b} Alessio Baldelli,^a Sam Thomas,^a Massimo Sgarzi,^c Matteo Gigli,^c Claudia Crestini,^c Fabrizio Cavani^{a,b} and Tommaso Tabanelli^{a,b}*

^a Dipartimento di Chimica Industriale “Toso Montanari”, Università di Bologna, viale del Risorgimento 4, Bologna, 40136, Italy;

^b Center for Chemical Catalysis - C³, Alma Mater Studiorum Università di Bologna, viale Risorgimento 4, Bologna, 40136, Italy

^c Ca' Foscari University of Venice, Department of Molecular Sciences and Nanosystems, Via Torino 155, Venice, 30172, Italy

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S1 Raw material and reagents

H₂SO₄ (72% solution), 1-Butanol (99,4%), Ethanol (99,8%), Fe₃O₄ (50-100nm), RuCl₃×3H₂O, Cellobiose (98wt.%), Glucose (99,8%), Xylose (99%), Xylitol (99%), Sorbitol (99%), Arabinose (99%), 1,2-PDO (99,5%), 1,3-PDO (98%), Glycerol (99,5%), Lactic acid (85%), Furfural (99%), HMF (99%), Levulinic acid (97%), Acetic acid (99,7%), Formic acid (95%), Phenol (97%), Eugenol (98%), Syringol (2,6-dimethoxyphenol) (98%), Guaiacol (99%), 4-propylguaiacol (99%), 5wt.% Ru/C were purchased by Merck-Sigma Aldrich.

Commercial microcrystalline cellulose Avicel® PH101, provided by Sigma-Aldrich, presents a particle size of 50 µm.

S2 Biomass pre-treatments

Before both characterization and fractionation reactions, pristine poplar wood samples were washed with water to remove soluble products (10 L of water was initially used for 1 Kg of biomass). After been oven dried overnight at 60 °C, the samples were processed through a ball milling procedure to reduce their size. In detail, 2 g of biomass was charged in a 60 ml stainless-steel reactor with 4 stainless-steel balls (2 12mm-diameter balls and 2 6mm-diameter balls), the milling time was maintained at 10 minutes in order to not overheat the sample (and avoid degradation) and to only reduce the size of the lignocellulose without affect cellulose crystallinity.

Lignocellulosic biomasses were at this point sized between 250-595 μm and stored in an open recipient for 24 h to reach an equilibrium with air humidity.

S3 Reductive Catalytic Fractionation Procedure

S3.1 Product separation

The reaction mixture was rapidly filtered to remove the carbohydrate pulp, while the filtrate solution is inserted in a separatory funnel in which spontaneous separation of aqueous and organic phase occurs at RT. Respectively, depolymerized hemicellulose-derived polyols are retained in the aqueous phase while lignin derived monomers and oligomers are retained in the organic phase. Samples of aqueous and organic phases were filtered using a 0.45 μm PTFE filter and kept for further characterizations (S5 and S6), consequently butanol is removed using a rotavapor and the viscous brown oil obtained was dried at 60°C overnight in order to calculate the weight of lignin derived products, and subsequently Lignin First Delignification Efficiency (LFDE). The pulp was washed with ethanol, to remove reaction mixture products adsorbed on the pulp surface, dried at 60°C overnight and weighted to calculate the overall LC conversion.

Conversion of LC and LFDE were calculated on the basis of the following equations:

$$\text{Conversion (\%)} = X_{LC} = \frac{m_{LCi} - (m_{LCf} - (m_{CATi} - m_{CATrec}))}{m_{LCi}} \times 100 \quad (1)$$

m_{LCi} = initial mass of Lignocellulose; m_{LCf} = final mass of cellulose pulp obtained after RCF; m_{cati} = initial mass of catalyst; $m_{cat rec}$ = mass of catalyst recovered after RCF

$$\text{Lignin First Delignification Efficiency (\%)} = \text{LFDE} = \frac{m_{\text{Lignin oil}}}{m_{LCi} \times \%_{\text{Lignin}}} \times 100. \quad (2)$$

m_{LCi} = initial mass of Lignocellulose; $\%_{\text{Lignin}}$ = weight percentage of lignin in the initial lignocellulose; $m_{\text{lignin oil}}$ = mass of lignin oil obtained through evaporation of the organic phase after RCF

S3.2 Catalyst recovery

When the magnetic catalyst was tested, the recovery procedure was performed on the post-reaction slurry. Conversely, when a heterogeneous solid catalyst was tested, the recovery procedure was performed on the solid pulp.

In the first scenario, the reaction mixture obtained after RCF process was put in agitation through a mechanical stirrer to obtain an ideal mixing of solids and both organic and aqueous phases. When the appropriate mixing was achieved, a neodymium magnet was placed in close proximity of the beaker wall. After 5 minutes the agitation was stopped, and the reaction mixture was poured in a second beaker while the magnet was kept stuck against the beaker wall (Figure S1); this way the



catalyst was retained from the reaction mixture, the operation was repeated a second time and the catalyst was washed with water and dried overnight at 60°C, the powder was then weighted to calculate the catalyst recovery efficiency (equation 3).

Figure S1: magnetic separation of the catalyst from the slurry obtained after the reaction.

When the catalytic tests were catalysed by Ru/C powder, the recovery procedure of the catalyst was performed by liquid-liquid extraction. In detail, the cellulose pulp containing the catalyst powder was washed with solvent mixtures (polar/apolar) reported in literature such as water/butanol³⁰ and decane/water. Substantially, relatively apolar Carbon is expected to be extracted by the apolar phase while the pulp remains at the bottom of the aqueous phase. To enhance the catalyst extraction, centrifugation and sonification of the slurry were also performed. After the extraction steps, the apolar solvent phase containing the solid catalyst was evaporated and the solid dried overnight at 60°C and weighted to calculate catalyst recovery efficiency (equation 3).

$$\text{Catalyst Recovery (\%)} = \frac{m_{\text{cat rec}}}{m_{\text{cat i}}} \times 100 \quad (3)$$

$m_{\text{cat i}}$ = initial mass of catalyst; $m_{\text{cat rec}}$ = mass of catalyst recovered after RCF

S4 Solid pulp and Biomass analysis

S4.1 Determination of water and ashes in pristine biomass

After completed biomass preparation (S1), both water and ashes content in the ball-milled biomass were evaluated gravimetrically after specific thermal treatments according to NREL procedures^{1,2}. In particular, a sample of biomass of nearly 3 g was oven-dried overnight at 105 °C and weighed. The procedure was repeated in triplicate and water content was calculated following the equation:

$$H_2O \text{ content (\%)} = \frac{m_{LCi} - m_{LCf}}{m_{LCi}} \times 100 \quad (4)$$

m_{LCi} = initial mass of Lignocellulose; m_{LCf} = final mass of dried Lignocellulose

Similarly, a sample of biomass of nearly 3 g was calcined following a specific temperature ramp: heating the sample at 10 °C/min up to 250°C, holding this temperature for 30 minutes, then 20 °C/min ramp up to 575 °C, keeping this temperature for 3 hours. After the last isotherm, the temperature is allowed to drop at 105°C and the samples is weighed. The procedure was repeated in triplicate and ashes content was calculated with the equation:

$$\text{Ash content (\%)} = \frac{m_{LCf}}{m_{LCi}} \times 100 \quad (5)$$

m_{LCi} = initial mass of Lignocellulose; m_{LCf} = final mass of calcined Lignocellulose

S4.2 Determination of structural carbohydrates and lignin in the pristine biomass and post-RCF pulps

Native lignocellulose biomasses (Figure S2, left) were compositionally analysed following a NREL LAP for standard biomass analysis³ performing a concentrated 72 wt% H₂SO₄ hydrolysis for 1 h at RT followed by dilute 4% H₂SO₄ hydrolysis in autoclave at 121 °C for 1 h. Structural components abundance was calculated on the basis of data collected by HPLC analysis (for the holocellulosic components) and by gravimetric calculations for Klason lignin content following equations:

$$\text{Cellulose content (\%)} = \frac{m_{(C6+C12)} \times \text{correction factor}}{m_{LCi}} \times 100 \quad (6)$$

$$\text{Hemicellulose content (\%)} = \frac{m_{(C5)} \times \text{correction factor}}{m_{LCi}} \times 100 \quad (7)$$

$$\text{Lignin content (\%)} = \frac{m_{LCf} (m_{cat i} - m_{cat rec})}{m_{LCi}} \times 100. \quad (8)$$

m_{C6} = mass of glucose present in the final solution; m_{C12} =mass of cellobiose in final solution; m_{C5} = mass of xylose, arabinose and galactose in the final solution

m_{LCi} = initial mass of lignocellulose; m_{LCf} = final mass of lignocellulose; m_{cati} = initial mass of catalyst; $m_{cat rec}$ = mass of catalyst recovered after RCF

The cellulose pulp obtained after RCF (Figure S2, right) was analysed through XRD analysis to verify its crystallinity and to confirm the absence (or negligible presence) of magnetic catalyst still trapped in the cellulose matrix (fig. S3).



Figure S2: Initial poplar wood sawdust and final cellulose pulp obtained through RCF with the fresh magnetic catalyst.

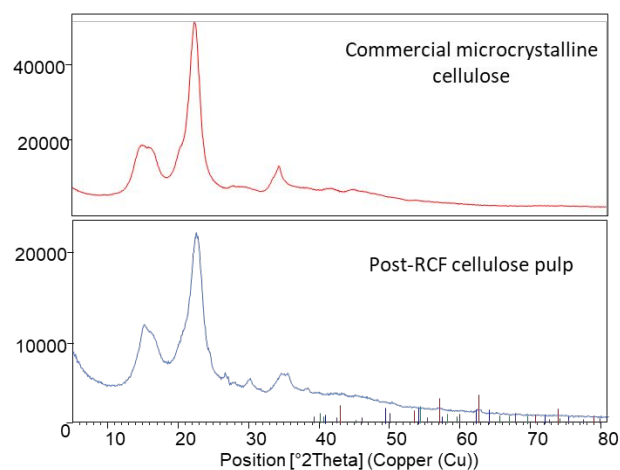


Figure S3: XRD patterns of the commercial microcrystalline cellulose (Avicel PH-101, top) and of a RCF derived cellulose pulp (bottom).

S5 Aqueous phase analysis

HPLC was performed to identify non-volatile products present in the aqueous phase, the analysis resulted in complex spectra from which different classes of products were identified. The solutions mainly included Oligomers, C₅₋₆ polyols and sugars (glucose, xylose, arabinose, sorbitol, xylitol, arabitol), C₂₋₃ hydrogenolysis products (ethylene glycol, glycerol, 1,2-Propandiol, Ethanol), Acetic Acid and Furans (Furfural, Furfuryl alcohol). The distribution of these classes of products are reported in Table S1.

Table S1: HPLC analysis of aqueous phases for group of products. Reaction conditions: 200°C, catalyst/LC=10wt.%, solvent/LC= 40L/Kg, P_{H₂}(RT)=30 bar. ^a yield expressed as mg of products/ mg of hemicellulose fraction.

	Oligomers (mg/g _{LC})	C ₅₋₆ polyols (mg/g _{LC})	C ₂₋₄ hydrogenolysis products (mg/g _{LC})	Acetic Acid (mg/g _{LC})	Total mg/g _{LC}	%products/ hemicellulose ^a
Blank	19.4	11.1	44.7	24	99.2	48.7
Ru/C	52.5	32.7	67.3	30.3	182.7	89
Ru/γFe ₂ O ₃	4.7	7.9	85	29.4	127.1	62.3

The most concentrated compound in the aqueous solutions is 1-butanol, the actual organic solvent used during RCF processes, because of its partial solubility in water. The concentration was found to be 7.3±0.4wt.%, which corresponds to its water solubility limit at saturation. Because of the substantial increase of the solution volume, due to the several washing steps necessary to recover the totality of the solid materials, this compound was found to be the most abundant in the aqueous media.

The detected C₅₋₆ polyols comprise xylitol, arabitol and in minor part sorbitol, while the C₂₋₄ hydrogenolysis products comprise shorter chain polyols (erythritol, glycerol, ethylene glycol) and their hydrogenation products (1,2-PDO, propanol, ethanol). Signals corresponding to oligomers were observed as well. Considering that the C₅ fraction amounts to 204 mg/g of biomass (in agreement with poplar wood characterization section of the main article), it is possible to establish a yield in aqueous products (reported in table S1). This yield should theoretically correspond to the hemicellulose conversion data reported in the main article and calculated on the residual hemicellulose in the final pulp, but in all cases this is not true. The reasons could be connected to the fact that if furanics (e.g. furfural or its hydrogenated forms) were produced in the process, it is

very likely that this fraction could have been removed from the aqueous phase and have been retained in the butanol fraction.

Nevertheless, it is noteworthy that the major species derived from hemicellulose are represented by C₂₋₄ polyols and that the relative abundance of this fraction raise with the presence of catalyst, meaning that the catalyst is involved in the hydrogenation of the hemicellulose derived products as well.

S6 Organic phase analysis

A Karl Fischer Volumetric Titrator HI933 of Hanna Instruments was used for the quantification of the water content in the organic phase media after the reaction. In particular 0.150 mL of solution was injected in the cell using Hydranal Medium K and Hydranal Composite 5K, supplied by Honeywell-Fluka, as solvent and titrant respectively.

S6.1 GPC

Organic phase solutions, sampled before the rotavap process, were analyzed through GPC analysis to determine the molecular weight of lignin oligomers produced during the treatment.

GPC was operated on a Shimadzu HPLC system by employing a PLgel 5 μ m MiniMIX-C column (250 x 4.6 mm). HPLC-grade DMSO containing 0.1% lithium chloride was used as eluent (0.5 mL/min, 70°C). Standard calibration was performed with polystyrene sulfonate standards (Sigma

Aldrich, 4.3–2600 kDa) and lignin model compounds (330–640 Da). A total of 8 standards were analyzed and interpolated with a 3rd order polynomial regression ($R^2 = 0.99$).

After lyophilisation, the samples were dissolved in HPLC-grade DMSO and filtered through a 0.45 μ m syringe filter prior to injection. Number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity value (\mathfrak{D}) of lignin oils analyzed by GPC were calculated following the equations:

$$M_n = \frac{\sum_i n_i M_i}{\sum_i n_i} \quad (9) \quad M_w = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i} \quad (10) \quad \mathfrak{D} = \frac{M_w}{M_n} \quad (11)$$

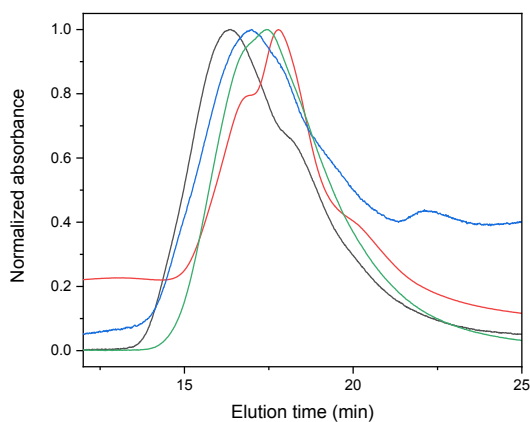


Figure S4: GPC profiles of lignin oils obtained in the absence of any catalyst (“blank”, 4 h treatment, black line) and in the presence of Ru/C (4 h treatment, red line) and RuO_x/ γ -Fe₂O₃ (2 h treatment, blue line; 4 h treatment, green line).

S6.2 Solvent recovery and phenolic monomer isolation: the fractional distillation and precipitation

40 mL of the organic phase after a RCF test in the presence of RuO_x/γ-Fe₂O₃ were sampled and processed through a fractional distillation to both quantitatively recover 1-butanol, which is recyclable for another RCF cycle, and isolate the lignin oil. In particular, firstly the 1-butanol-water azeotrope was distilled at 89 °C at atmospheric pressure (11 mL of 1-butanol and 4.5 ml of water), this way confirming the presence of water in the organic phase (ca. 14 wt.%); then a slight vacuum was applied to completely distill 24 ml of 1-butanol. The obtained lignin oil was then weighted and further processed via fractional precipitation, firstly dissolving the oil in 30 mL of toluene and then adding *n*-heptane until a volumetric ratio between heptane and toluene of 10/7 was reached. This way the precipitation of the more polar oligomers was promoted, obtaining a solution with only the lighter fraction of the lignin oil. The structure of monomers, as well as their quantification, was conducted by GC-MS, Agilent Technologies 6890 GC equipped with an Agilent HP-5 capillary column (30 m × 250 μm × 1.05 μm, T_{inj}=280°C; split ratio: 30:1, He flow: 0.5 mL min⁻¹) and an Agilent Technologies 5973 mass analyzer. The temperature ramp was composed of an initial 2 min isothermal step at 50 °C, then a 10 °C/min ramp up to 130 °C, 2 min isothermal step, 20 °C/min ramp up to 280 °C, and a final isothermal step of 5 min. Monomers yields were calculated following equation 12:

$$\text{Monophenolics (\%wt)} = \frac{\sum m_{\text{monomers}}}{m_{\text{lignin } i}} \quad (12)$$

Whenever possible, commercially available standards were used to calibrate the response factor in the appropriate concentration range, whereas *n*-octane was used as Internal Standard. Monomer identification was performed using the GC-MS internal database, by comparison with the literature, and, whenever possible, by comparison to authentic commercial samples.

S7 Catalyst characterization

S7.1 TPR and CO chemisorption

Considering TPR analysis results (Figure 3 of the main article) and performing the integration of the relevant peaks, it was possible to calculate an overall H₂ consumption and, therefore, a reduction grade of the material (comparing the measured consumption with the theoretical consumption due to the reduction of RuO₂ to metallic Ru and from maghemite to magnetite as described in the equation 1 of the main text). As shown in Table S2, the bare support, the fresh and the spent catalysts show a reduction grade of above 90%. The loss in wt. % of material, which is not reduced during the TPR analysis, is probably constituted by hematite formed as undesired product during the calcination of the catalyst precursor.

Table S2: γ -Fe₂O₃, fresh and spent RuO_x/ γ -Fe₂O₃ reduction grade related to the main peak of H₂-TPR, the conversion of maghemite to magnetite and the reduction of RuO₂ to Ru⁰ has been considered. In the case of the spent catalyst a 99.9% of reduction grade has been obtained by considering the complete reduction of RuO₂ to Ru⁰ as well as the co-presence of both magnetite and maghemite in the support in a weight ratio of 55:45 respectively.

Cat.	g cat. loaded	mol cat.	H ₂ consumed (mol)	Theoretical H ₂ consumption (mol)	Reduction grade (%)
γ -Fe ₂ O ₃	0.2025	12.69×10 ⁻⁴	3.97×10 ⁻⁴	4.23×10 ⁻⁴	93.86
Fresh RuO ₂ / γ -Fe ₂ O ₃	0.1365	6.65×10 ⁻⁵ (RuO ₂) + 7.99×10 ⁻⁴	3.67×10 ⁻⁴	3.99×10 ⁻⁴	91.8
Spent catalyst	0.1105	5.55×10 ⁻⁵ (RuO ₂) + 3.23×10 ⁻⁴	2.08×10 ⁻⁴	2.08×10 ⁻⁴	99.9

Table S3: CO-chemisorption results obtained for fresh 5 wt.% RuO_x/ γ -Fe₂O₃ previously reduced with H₂ at 180 °C. ^a calculated by dividing the μ mol of adsorbed CO for the μ mol of Ru; ^b calculated as reported in ref. 4; ^c calculated as reported in ref. 5.

Sample	Adsorbed CO (μ mol/g)	Metal dispersion (%) ^a	SSA metal (m ² /g) ^b	Estimated average particle sizes (nm) ^c
5 wt.% RuO _x / γ -Fe ₂ O ₃	26.6	5.4	19.7	18.6

S7.3 Porosimetry

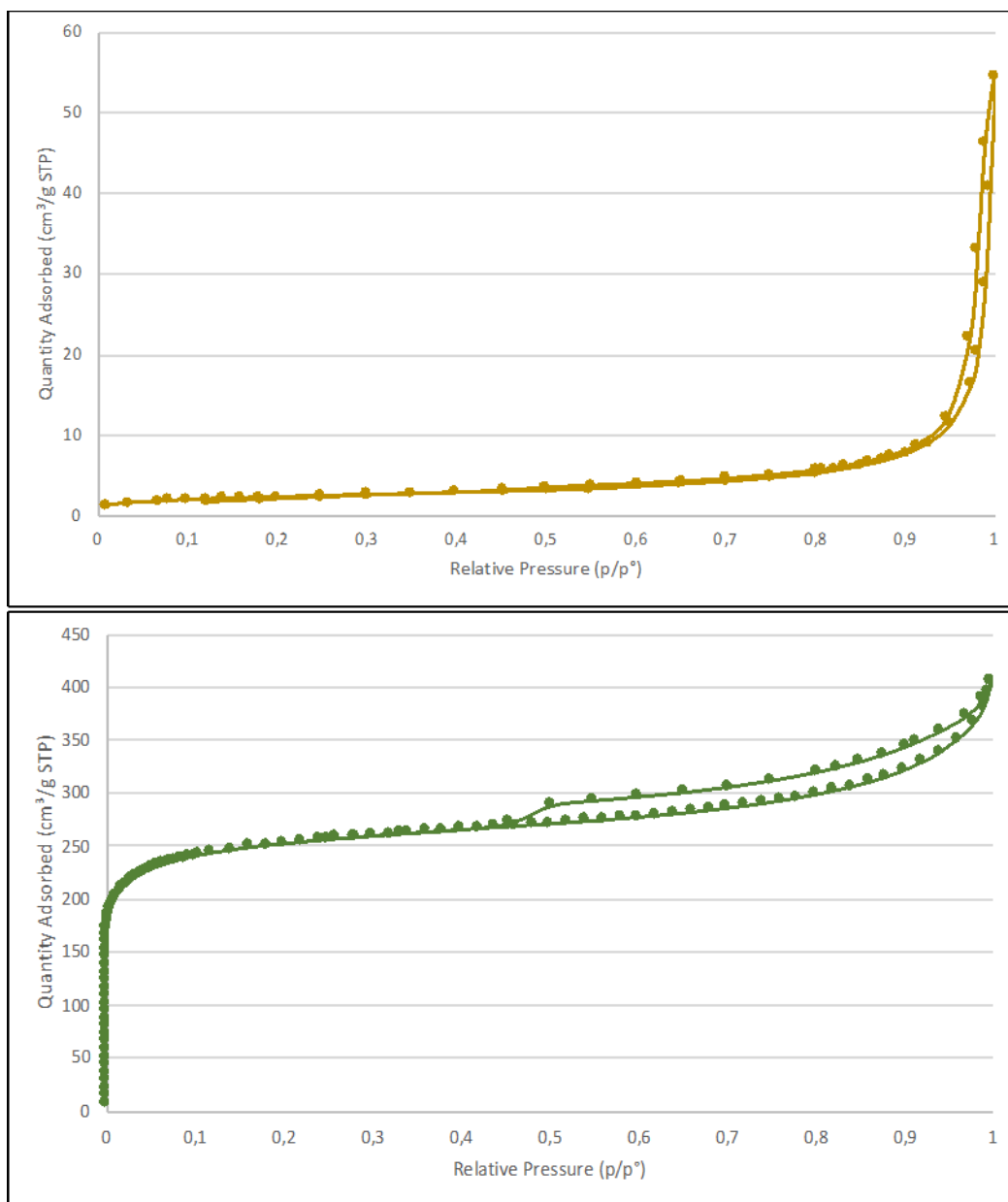


Figure S5: Physisorption curves of RuOx/γ-Fe₂O₃ (top) and Ru/C (bottom) catalysts

S7.4 TEM and FEG-SEM

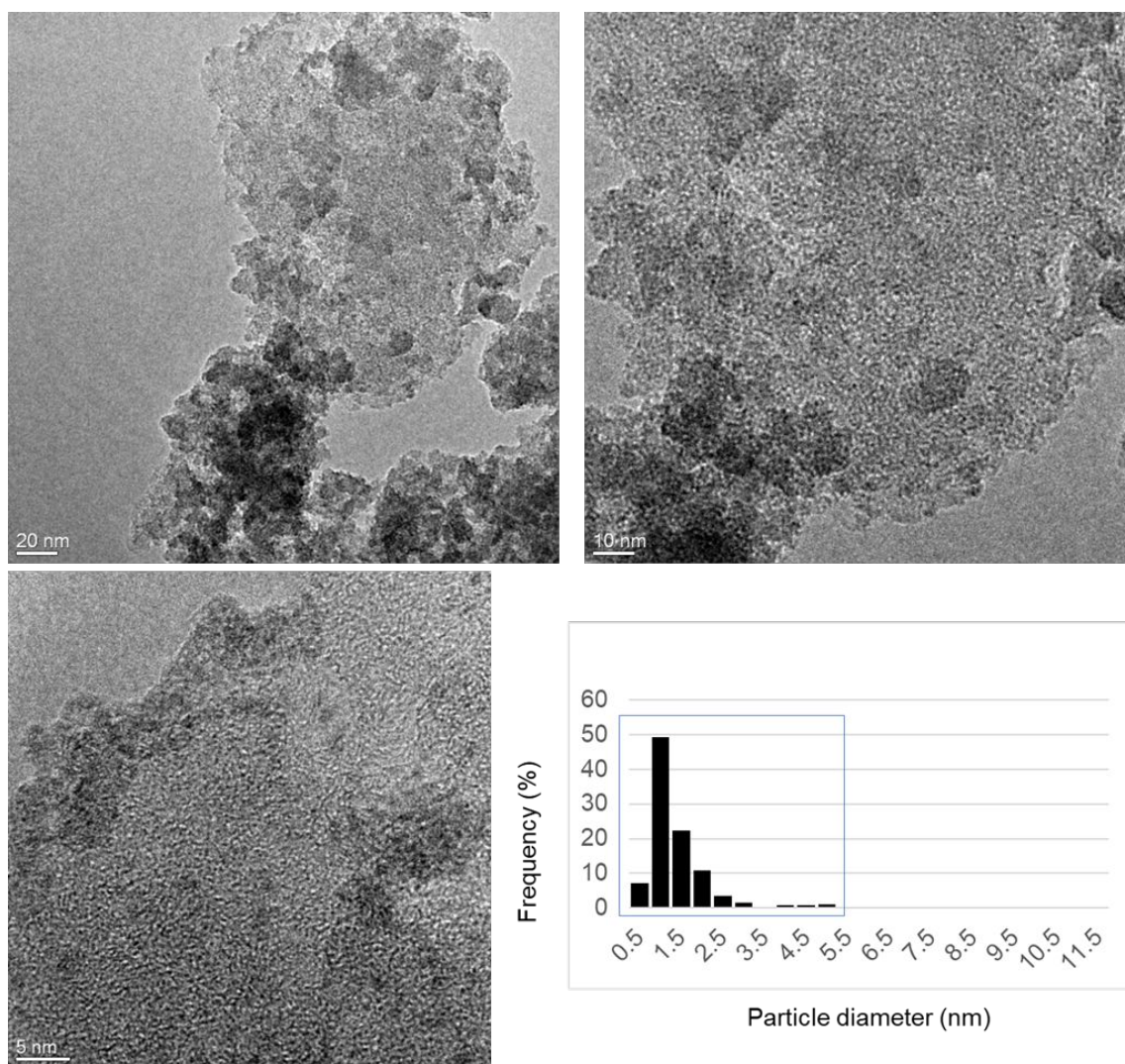


Figure S6: TEM micrographs and corresponding histograms of Ru particle size distributions for the fresh Ru/C catalyst.

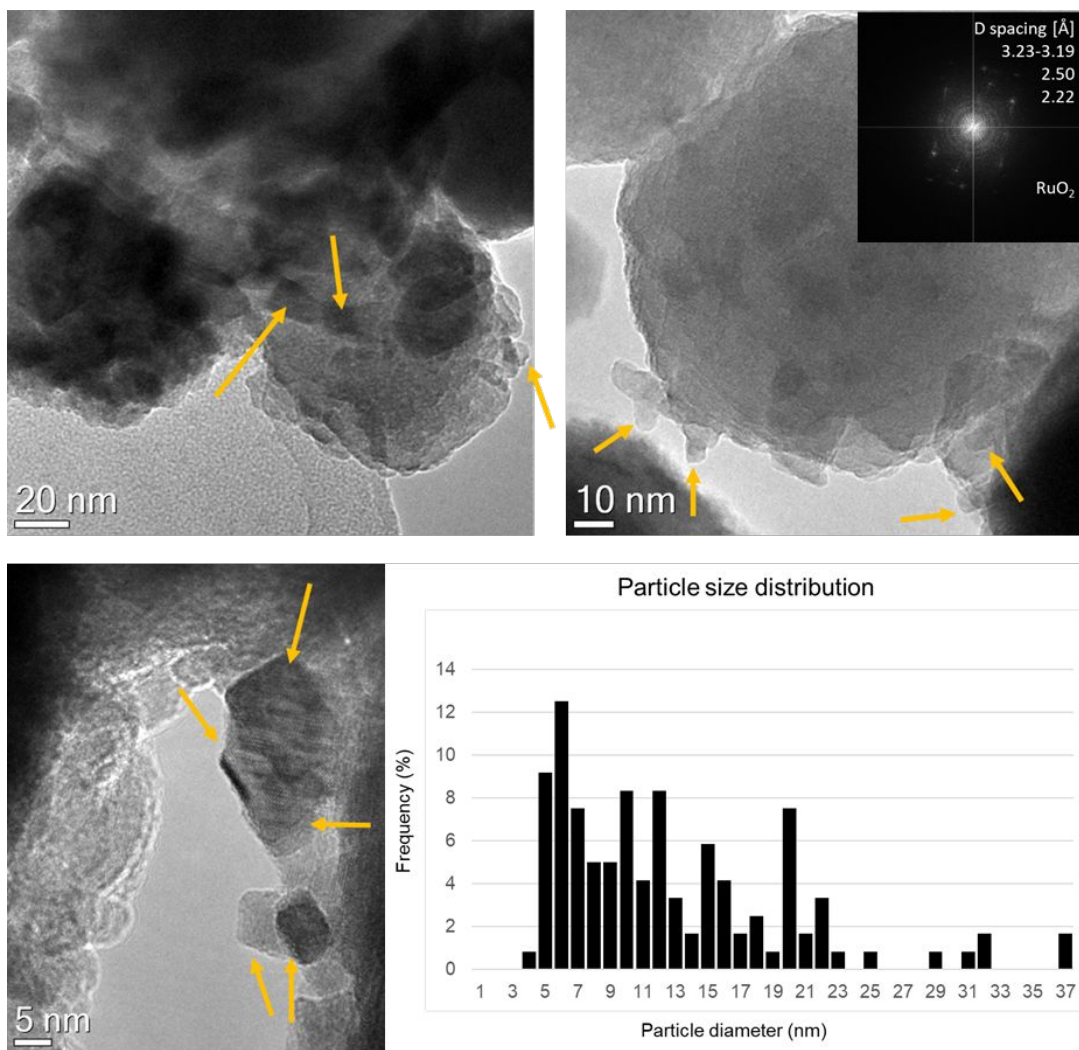


Figure S7: TEM micrographs and corresponding histograms of RuO₂ particle size distributions for the fresh RuOx/γFe₂O₃ catalyst. Yellow arrows highlight the presence of RuO₂ particles of cubic shape.

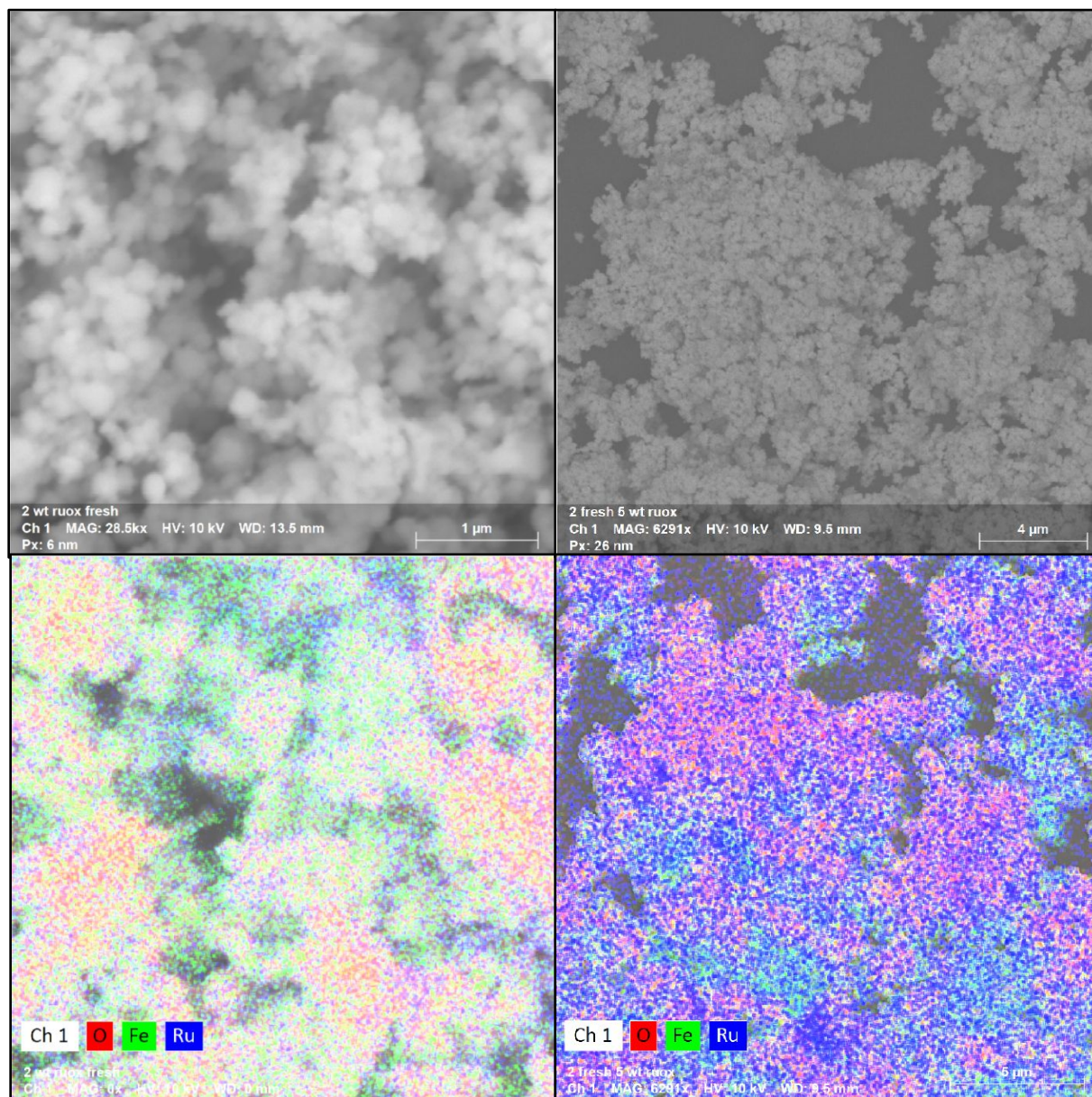


Figure S8a: FEG-SEM micrographs (top) with elemental mapping details (bottom) for the fresh RuOx/γFe₂O₃ catalyst at two different magnifications.

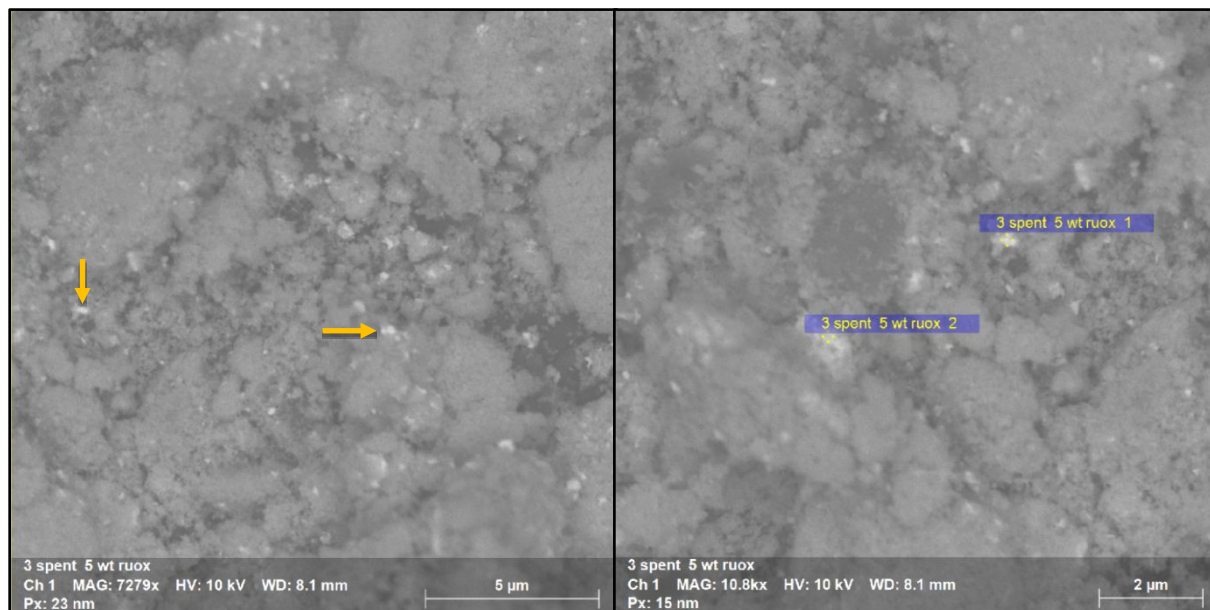


Figure S8b: FEG-SEM micrographs with elemental analysis for the spent RuOx/γFe₂O₃ catalyst at two different magnifications. The presence of metallic ruthenium particles was confirmed by spot elemental analysis (brighter particles also highlighted by yellow arrows).

S7.5 TGA of the spent catalyst

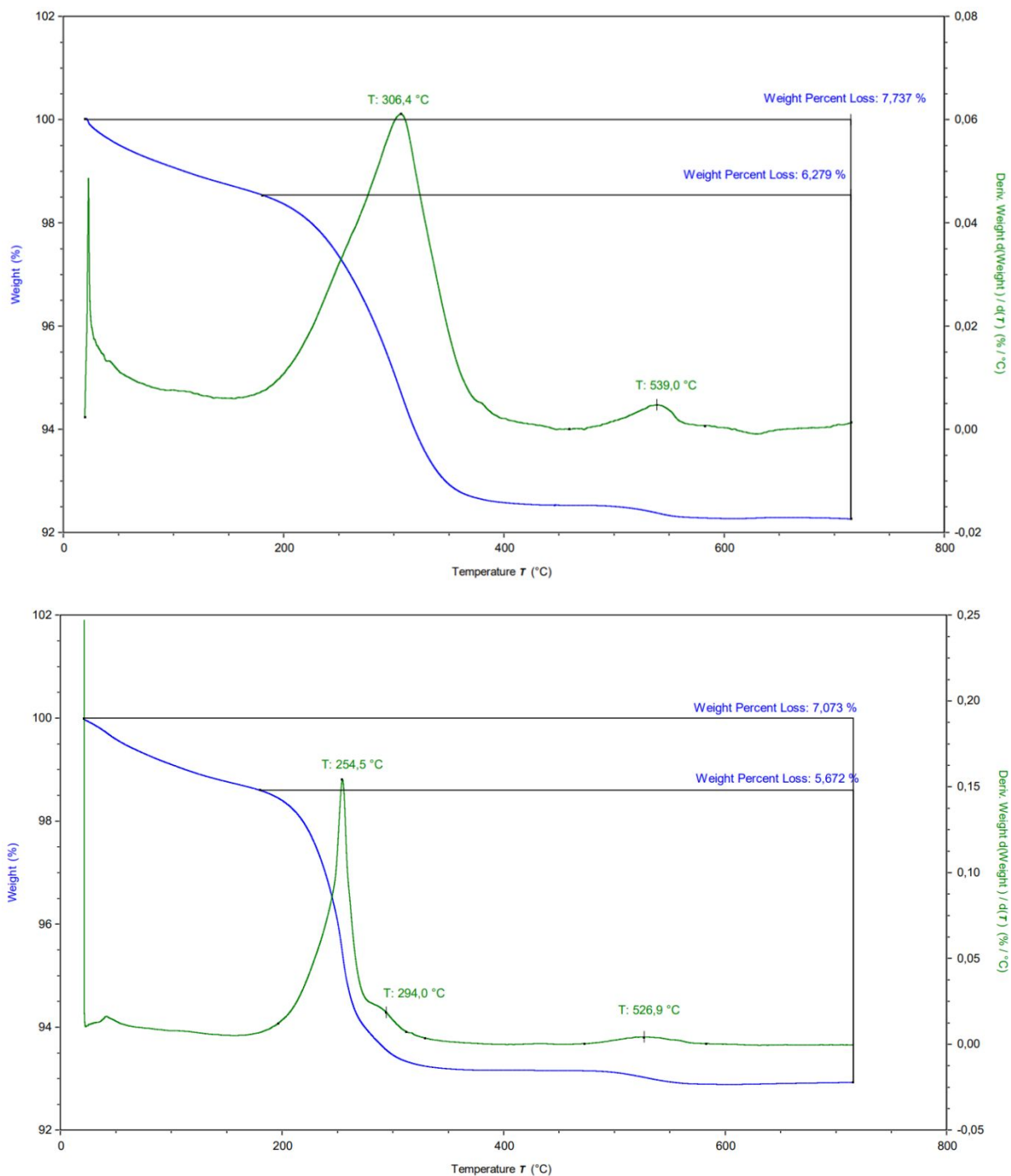


Figure S9: TGA profile of the spent catalyst after three RCF cycles, performed either in nitrogen flow (top) or in air (bottom). Weight loss (blue) and the derivative curve of the weight loss (green) are plotted in function of the temperature.

S8 Leaching tests

MP-AES analysis was performed on the both the organic and aqueous phase to determine the leaching of the catalyst during the reaction conditions. The results show no leaching in the organic phase (not shown), while the leaching in the aqueous phase, in terms of either ppm in solution (after dilution for the analyses) and % of element leached compared to the initial mass of catalyst loaded in the reactor, are reported in Table S3.

Table S4: MP-AES analysis on liquid phase obtained after every recycle tests on the RuOx/ γ -Fe₂O₃ catalyst in the RCF of poplar wood. Reaction conditions: 200°C, catalyst/LC = 10wt.%, solvent/LC = 40L/Kg, P_{H₂}(RT) = 30 bar.

	Fe ppm (373.713 nm)	Ru ppm (366.136 nm)	Fe (mg)	%Fe leached	Ru (mg)	%Ru leached
I cycle	2.31	0.06	0.59	0.70	0.015	0.24
II cycle	1.51	0.03	0.44	0.57	0.009	0.15
III cycle	1.5	0.03	0.24	0.48	0.005	0.13

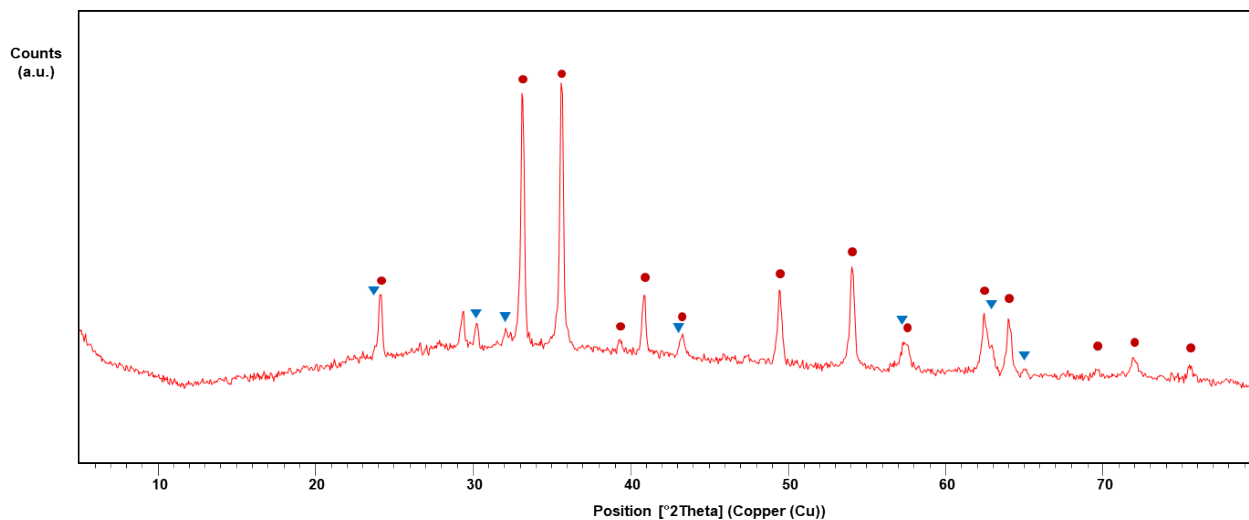


Figure S10: XRD pattern of the residual solid after the calcination of the cellulose pulp obtained after the third cycle of RCF. Hematite ($\alpha\text{-Fe}_2\text{O}_3$, red circle), maghemite ($\gamma\text{-Fe}_2\text{O}_3$, blue triangles).

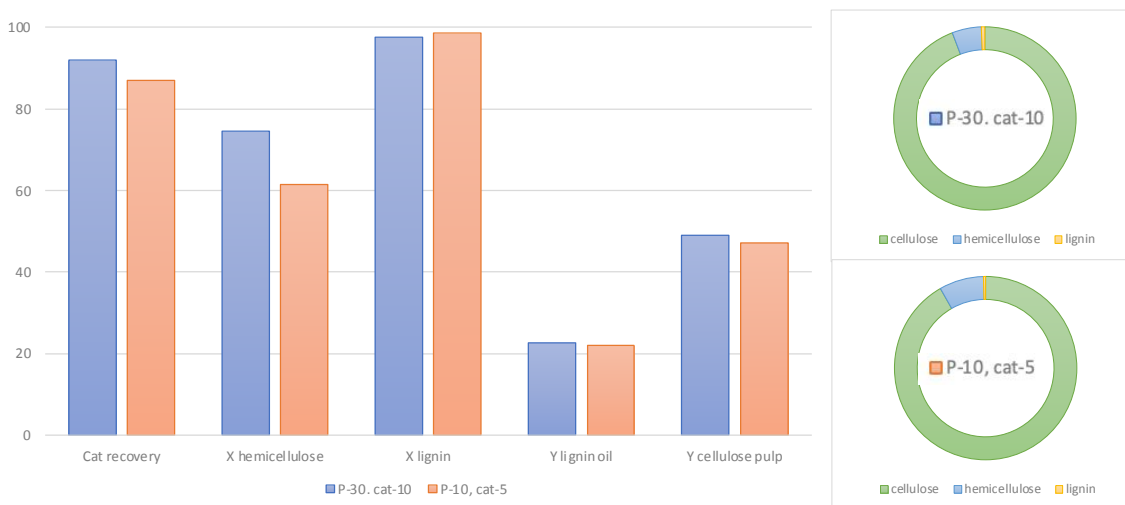


Figure S11: Catalytic tests for the RFC of Poplar wood using a 5wt.% $\text{RuO}_x/\gamma\text{-Fe}_2\text{O}_3$. Reaction conditions: 200 °C for 4 hours reaction time, with 1-butanol- H_2O (50-50) mixture as solvent. “P-30, cat-10” results obtained with 30 bar of H_2 loaded at RT and 10wt.% of catalyst compared to the loaded biomass; “P-10, cat-5” 10 bar of H_2 loaded at RT and 5wt.% of catalyst compared to the loaded biomass.

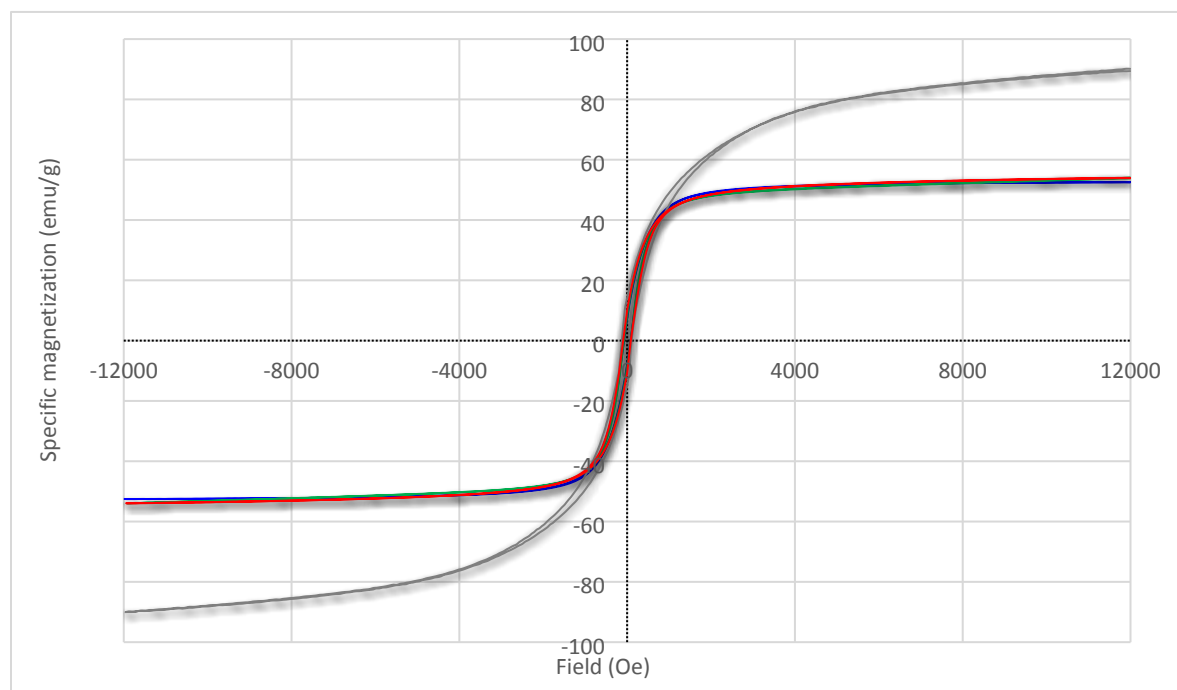


Figure S12: Magnetization curves of pure maghemite (blue), fresh 5 wt.% RuOx/γ-Fe₂O₃ (green), spent 5 wt.% RuOx/γ-Fe₂O₃ catalyst after one reaction cycle (grey) and spent recycled catalyst (i.e. after two reaction cycles, in red).

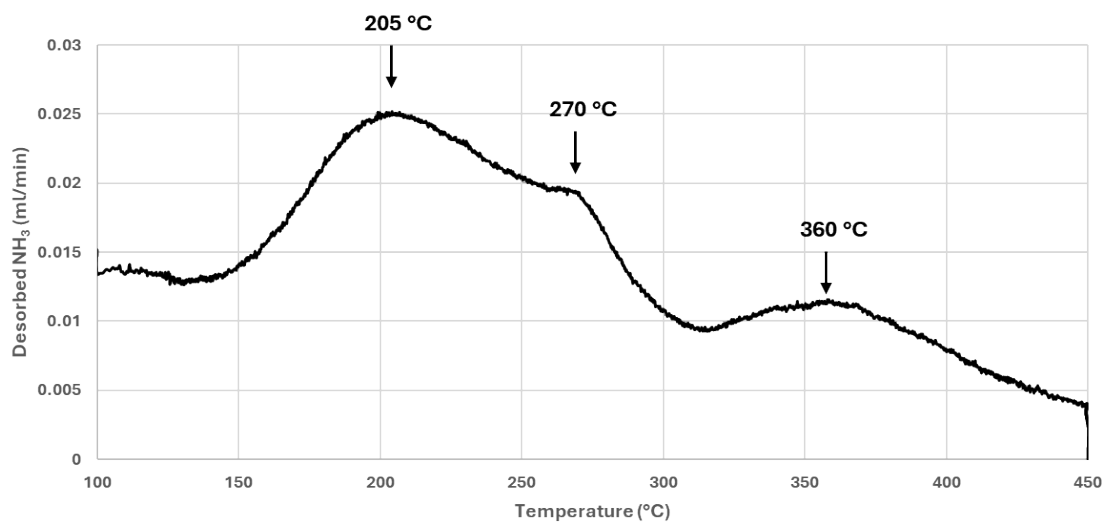


Figure S13: NH₃-TPD profile for fresh 5 wt% RuOx/γ-Fe₂O₃.

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