1. Materials and Methods

1.1. Chemicals

Unless stated otherwise, all commercially available chemicals were used without further purification. Vanillin (99%), vanillin-\textsuperscript{13}C\textsubscript{6} (99.2 \% \textsuperscript{13}C), Cu(II)SO\textsubscript{4}·5H\textsubscript{2}O (98%), were purchased from Sigma Aldrich. 1-octanol (99.5\%) was purchased from TCI. Acidic aqueous stock solutions were prepared as follows: 5 mM aqueous solutions of Cu(II)SO\textsubscript{4}·5H\textsubscript{2}O were obtained by dissolving 100 mg of salt in 80 ml of distilled water. pH was adjusted to 1 by addition of 440 \(\mu\)l of concentrated H\textsubscript{2}SO\textsubscript{4} 96%. Kraft lignin \textit{Indulin AT} (softwood, water content 3.5 wt.\%) was provided by Ingevity (5255 Virginia Ave. North Charleston, SC, US). Lignin content was declared by the supplier. Water content was determined by Karl-Fischer titration. Lignins with particle size < 300 \(\mu\)m were obtained by sieving through metallic analytical sieves with a mesh spacing of 300 \(\mu\)m.

1.2. Parameter screening

The set-up used in the current study was designed to combine mild conversion conditions (170 °C, 1 MPa) with an efficient production of vanillin. Experiments were carried out under oxygen atmosphere and pure molecular oxygen: inexpensive, effective, and environmentally friendly reactant. In addition to oxygen, oxidation catalyst, CuSO\textsubscript{4}, was used, known from literature to accelerate liquid-phase oxidations with molecular oxygen. p-Xylene was used as the organic solvent in some runs for comparative purposes to study the scavenging effect of the octanol. A summary of runs (\(R_{a} - R_{o}\)) for the tested parameters can be found in Table 1. A standard set of process parameters was applied as an experimental starting point. The
aim was to provide a point of reference to the innovative in-situ product extraction procedure by comparing the results to the large body of studies dealing with the selected area of lignin valorization, i.e. monophasic acid-catalyzed oxidation of technical lignins in the presence of oxygen and at low pH. The following standard conditions were chosen: \( T = 170 \, ^\circ C \), \( p = 0.5 \, \text{MPa} \) of initial oxygen pressure (at 25 \( ^\circ C \)), \( t = 40 \, \text{minutes} \) reaction time, 5 ml water with 0.1 M \( \text{H}_2\text{SO}_4 \) and 5 mM Cu(II) as \( \text{CuSO}_4 \cdot 5\text{H}_2\text{O} \), 5 ml 1-octanol, and 0.1 g of lignin (corresponding to 10 g/L of lignin based on the total liquid volume). From this set of conditions, only one parameter was changed at a time in each successive test to allow for a meaningful comparison.

1.3. Batch reactor

For the solvothermal reactions, a batch reactor (Premex AG, Lengnau, Switzerland; model Varioso), was used. The reactor has the following specifications: nominal volume 60 ml, maximum operating temperature 240 \( ^\circ C \), maximum pressure 30 MPa (safety valve opens at 8 MPa). The reactor is made of alloy Nr. 1.4980 HP. Given the corrosive conditions, a polytetrafluoroethylene (PTFE) liner with an internal volume of 46 ml was used to accommodate the reaction medium. The reactor was placed in a heating block, which in turn sat on a hot plate. Mixing was performed with a PTFE magnetic stirrer by means of a magnetic plate. Pressures were read by means of a pressure gauge, class 1. Temperature inside the reactor was measured by a Pt100 type sensor coupled to the hot plate.

1.4. Oxidation experiments

For the two-phase experiments, 5 ml of aqueous solution and 5 ml of octanol were added into the reactor. Alternatively, for the one-phase experiment, 10 ml of aqueous solution were used. 100 mg of powdered lignin were then added, resulting in a lignin concentration of 10 g/L based on the total liquid volume. The reactor was sealed, purged 6 times with oxygen, and then filled to a pressure of 0.5 MPa. The mixture was heated to 170 \( ^\circ C \) at a rate of 8 K/min. After keeping the mixture at (170 \( \pm 3 \) \( ^\circ C \)) for the desired time (at this temperature, a pressure of \( \approx 1.1 \, \text{MPa} \) was measured in the two-phase system; when only water was employed, a pressure \( 1.2 \, \text{MPa} \) was measured), the reactor body was immersed in a water bath for approx. 20 minutes. When the temperature of the reaction medium reached 30 \( ^\circ C \), the remaining pressure (\( \approx 0.4 \, \text{MPa} \)) was released, and the reactor was opened. No loss of liquid volume was detected. 1 ml of each phase (water and octanol/p-xylene phase) was taken and stored at \( -20 \, ^\circ C \). No losses in yields were detected upon thawing.

1.5. Analytical methodology

1.5.1. Sample preparation

Samples from each phase were directly taken from the reaction mixture with no intermediate extraction step. In addition, we used UHPLC coupled with a high-resolution mass spectrometer to detect and quantify vanillin, instead of the more common GC-FID method. This allowed us to employ isotopically enriched
vanillin-\textsuperscript{13}C\textsubscript{6} as internal standard, and yields were obtained against the corresponding calibration curve (Figure S1). The retention time and elution profile of vanillin and vanillin-\textsuperscript{13}C\textsubscript{6} are virtually identical. Matrix effects affecting elution and ionization are effectively compensated by the internal standard. Thus vanillin-\textsuperscript{13}C\textsubscript{6} is the ideal internal standard for vanillin quantification by having the same chemical properties and a mass difference of 6 atomic units (\textsuperscript{13}C in the benzene ring).

1.5.2. UHPLC-HRMS

The chromatographic separation of vanillin took place in a Thermo Scientific Dionex Ultimate 3000 Series RS system (Thermo Fisher Scientific, Switzerland). The system contained a pump, a column compartment, and an auto-sampler. Mobile phase A (1 vol.% acetonitrile [ACN], 1 vol.% methanol [MeOH], and 0.2 vol.% formic acid [HCOOH] in high purity water) and mobile phase B (99 vol.% ACN in high purity water) were used at the flow rate of 0.250 ml/min. The following gradient was applied: 1 vol.% B (0-2 min), from 1 to 99 vol.% B (2-12 min), and 99 vol.% B (12-16 min) followed by re-equilibration (16-20 min). 2 \( \mu \)l of the prepared solutions was injected. Separation of the analytes was achieved with an ACQUITY UPLC C-18 VanGuard\textsuperscript{TM} pre-column and column (150 mm x 2.1 mm x 5 mm, particle size 1.7 \( \mu \)m) from Waters (Switzerland). The temperature of the columns was 50 °C. Heated electrospray ionization (ESI, 3.5 kV spray voltage) in positive mode was used for the ionization. Data acquisition was performed using a Thermo Scientific Q-Exactive\textsuperscript{TM} hybrid quadrupole-orbitrap mass spectrometer controlled by Xcalibur 4.1 software (Thermo Fisher Scientific, Switzerland). Mass spectra were acquired in full scan mode with an isolation window of 1 m/z from 50-750 m/z. The resolution was set to 70,000.

1.5.3. Quantification of vanillin

All samples for vanillin quantification were prepared by pipetting on an analytical balance. Nine stock solutions of vanillin in MeOH, ranging from 1 to 780 \( \mu \)M, were prepared, containing around 10 \( \mu \)M vanillin-\textsuperscript{13}C\textsubscript{6}. As can be deduced from Fig. S2 the retention time and elution profile are virtually identical. Thus \textsuperscript{13}C-vanillin is the ideal internal standard for vanillin quantification by having the same chemical properties and a mass difference of 6 atomic units. Each measurement was repeated three times. The resulting LC area ratios were then extracted and plotted against the corresponding concentration ratios (Fig. S1). The non-weighted calibration curve shows the following statistic: intercept = 0.22 ± 0.02; slope = 0.55 ± 0.01; Pearson’s R\textsuperscript{2} = 0.999. To check the quality of the calibration curve, known amounts of vanillin were measured and the corresponding concentrations calculated. All Quality Control samples (QCs) were within 5 CV\% (coefficient of variation). The LC area ratios for all experimental samples after dilution with the IS solution fall within the confidence band (95\% level) of the calibration curve.

Prior to UHPLC-HRMS measurements of the samples, approximately 10 \( \mu \)l of filtered solution (0.22
µm PTFE filter, Chromacol) obtained after the solvothermal experiment were pipetted on balance with 80 µl volume of methanol and 10 µl of an internal standard stock solution of vanillin-^{13}C_6 (100 µM). Each measurement was performed in triplicate. The corresponding coefficient of variation (CV%) was always below 5%. The calibration curve (Figure S1) was used to determine vanillin concentration in the experimental samples. LC areas were extracted and the corresponding concentration was determined by means of the calibration curve. Vanillin yields (Y_v) were calculated on the dry lignin basis. The run at standard conditions (R_a) was repeated three times: the inter-experimental standard error of the mean was 2% and 2.4% for organic and water phase, respectively. Because all experiments were run using the same instrumentation and analytical procedures, one can assume the same uncertainty to hold also for the other calculated yields. The error on vanillin yields are depicted together with the experimental results. To evaluate the stability of vanillin after the solvothermal experiments, samples of reaction mixtures were stored at room temperature, and aliquots were taken at given times and measured. According to Fig. S3, vanillin is stable at room temperature for at least one week.

![Calibration curve for vanillin quantification](image)

Figure S1: Calibration curve for vanillin quantification. Area ratio fitted linearly to the concentration ratio of vanillin and internal standard. Weighting as follows: y-error standard deviation of triplicate measurements, x-error was set to zero. Statistical details: intercept = 0.02±0.01; slope = 0.48±0.01; Pearson’s R^2 = 0.999. All Quality Control samples (QCs) within 5% CV. 95% confidence band represented as gray area. Note: error bars in y-direction are too small to be visible.
Figure S2: Extracted ion chromatogram (XIC) of vanillin and $^{13}$C-vanillin. The traces are extracted using theoretical mass of the component $\pm$ 1 ppm. The retention time and elution profile are virtually identical. Thus $^{13}$C-vanillin is the ideal internal standard for vanillin quantification by having the same chemical properties and a mass difference of 6 atomic units.
Figure S3: Variation in the LC-MS/MS area over time for the vanillin signal at acidic conditions. Above: water phase; below: octanol phase.