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ARTICLE

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Limited life cycle and cost assessment for the bioconversion of lignin-derived aromatics into adipic acid

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Abstract

Lignin is an abundant and heterogeneous waste byproduct of the cellulosic industry, which has the potential of being transformed into valuable biochemicals via microbial fermentation. In this study, we applied a fast-pyrolysis process using softwood lignin resulting in a two-phase bio-oil containing monomeric and oligomeric aromatics without syringol. We demonstrated that an additional hydrodeoxygenation step within the process leads to an enhanced thermochemical conversion of guaiacol into catechol and phenol. After steam bath distillation, *Pseudomonas putida* KT2440-BN6 achieved a percent yield of *cis*, *cis*-muconic acid of up to 95 mol% from catechol derived from the aqueous phase. We next established a downstream process for purifying *cis*, *cis*-muconic acid (39.9 g/L) produced in a 42.5 L fermenter using glucose and benzoate as carbon substrates. On the basis of the obtained values for each unit operation of the empirical processes, we next performed a limited life cycle and cost analysis of an integrated biotechnological and chemical process for producing adipic acid and then compared it with the conventional petrochemical route. The simulated scenarios estimate that by attaining a mixture of catechol, phenol, cresol, and guaiacol (1:0.34:0.18:0, mol ratio), a titer of 62.5 (g/L) *cis*, *cis*-muconic acid in the bioreactor, and a controlled cooling of pyrolysis gases to concentrate monomeric aromatics in the aqueous phase, the bio-based route results in a reduction of CO_2-eq emission by 58% and energy demand by 23% with a contribution margin for the aqueous phase of up to 88.05 euro/ton. We conclude that the bio-based production of adipic acid from softwood lignins brings environmental benefits over the petrochemical procedure and is cost-effective at an industrial scale. Further research is essential to achieve the proposed *cis*, *cis*-muconic acid yield from true lignin-derived aromatics using whole-cell biocatalysts.

KEYWORDS

catechol, *cis*, *cis*-muconic acid, fast-pyrolysis, lignin, *Pseudomonas putida* KT2440

1 | INTRODUCTION

Lignin is nature's second most abundant polymer and displays a largely unexploited renewable resource for value-added bioaromatics. High-value applications for lignin are currently initiated (UPM, Finland; Stora Enso, Finland. For instance, processed lignin applies as a polymer additive (e.g., for polyolefins such as polypropylene and polyethylene), an alternative to bitumen, a component of cement, a carbon fiber, a soil improver, and a phenolic resin and adhesive (e.g., for coating applications) (de Wild, Huijgen, & Gosselink, 2014). By using lignin as a dispersant for dyes and pesticides, it accounts already for 1×10^5 tons of the compound. By the kraft process also known as the sulfate process, wood is converted into pulp, consisting of cellulose, the main component of the paper. Black liquor containing lignin, hemicellulose degradation products, and other extractives is a waste stream when not used materially on a large scale (Tuck, Pérez, Horváth, Sheldon, & Poliakoff, 2012). At present, the pulp and paper industry produces around 50 million tons of lignin per year, 98% of which is burned to generate heat and steam (Azadi, Inderwildi, Farnood, & King, 2013; Gosselink, de Jong, Guran, & Abächerli, 2004). Examples of lignin recovered from black kraft liquor (which accounts for 85% of total lignin production) offered to the market are Lignoboost™ (Innventia, Sweden) and IndulinAT™ (MeadWestvaco Corporation, Richmond, VA). Lignoboost™ precipitates by the addition of CO_2 to acidify the solution to off-load the recovery boiler. After dewatering using a filter press, the precipitate is washed two times with H_2SO_4 acidified water to minimize the sodium content in the final lignin product. In total, Domtar, Stora Enso, and LignoDemo AB (Sweden) produce 0.83×10^5 tons per year. All three plants use the Valmet process (Finland). The technology originates from Innventia and Chalmers University, Sweden. IndulinAT™ from linerboard-grade pulp precipitates by acid hydrolysis from kraft black liquor to remove sodium and the hemicellulose. For the past 60 years, MeadWestvaco commercialized IndulinAT™. The total production is about 3×10^4 tons per year. Another interesting resource for lignin is the organosolv pulping of lignocellulosic biomass, developed at the demonstration scale (Lignol, Canada; Chempolis, Finland; CIMV, France). High purity of lignin can be fractioned with a limited amount of residual carbohydrates and minerals (Laurichesse & Averous, 2014). Moreover, lignin has an intrinsic energy value of approximately 26.7 MJ/kg, which corresponds to a heating value of 62 euro/ton from gas and 193 euro/ton from crude oil (Tomani, 2010; Trading Economics, Portugal; Natural Gas Australia, Australia).

To increase the use of lignin, the Energy Research Center of the Netherlands (now ECN part of TNO, the Netherlands) developed the lignin biorefinery approach (LIBRA) fast-pyrolysis process using bubbling fluidized bed reactor technology (de Wild et al., 2014). The total mass of pyrolysis products can compose up to 100% of the organic carbon (Bridgwater, 2012; Butler, Devlin, Meier, & McDonnell, 2011; Yildiz, Ronsse, van Duren, & Prins, 2016). In addition to the two-phase lignin bio-oil (45 wt/wt%), gas (20 wt/wt%), and char (35 wt/wt%) accumulate (Figure 1). Lignin bio-oil exists out of oligomeric (20 wt/wt%) and monomeric (10 wt/wt%) phenolic compounds, the latter are also dissolved in water (15 wt/wt%) (de Wild et al., 2014). Regarding the two-phase bio-oil, the phenolic compounds forming the organic phase can be used as a suitable substitute for the petrochemical-based phenol as a starting material for the synthesis of phenol–formaldehyde resins. These resins are widely used in the wood industry, for example, for plywood, wood laminates, and particle board. However, the presence of the methoxy-phenols such as syringol, guaiacol, and catechol causes a resin having diminished properties as lower reactivity and a higher viscosity due to their limited cross-linking reactivity with formaldehyde. As shown in this study, syringol is not present in bio-oil from softwood kraft and organosolv lignin (de Wild, Huijgen, Kloekhorst, Chowdan, & Heeres, 2017), and the application of hydrodeoxygenation reduces the concentration of guaiacol (Ben

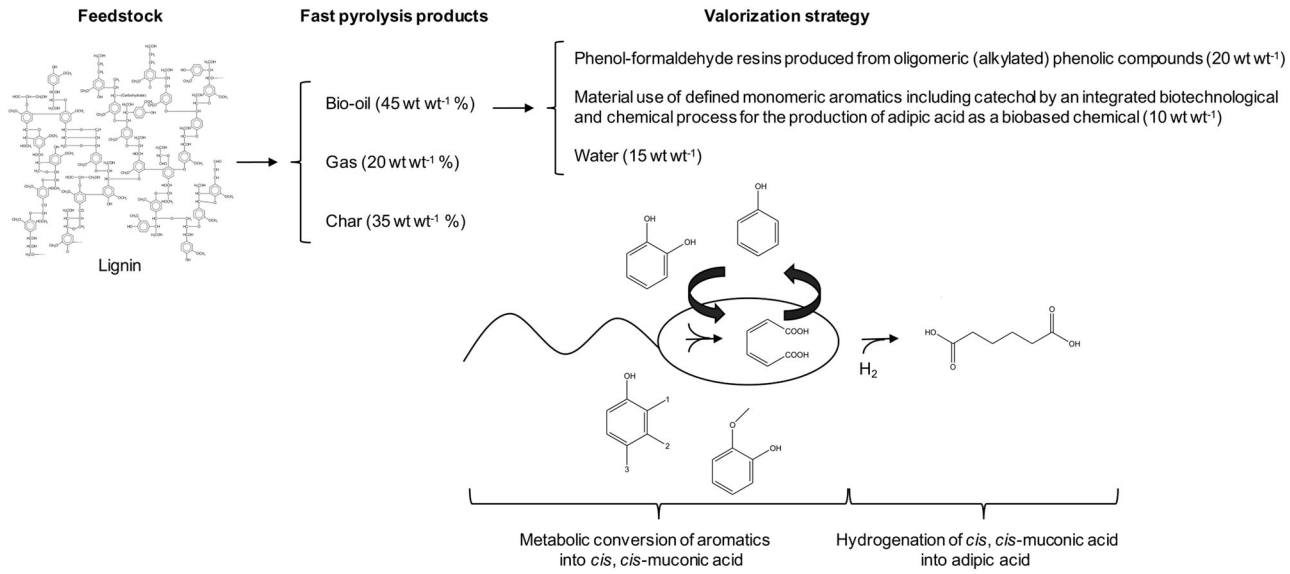

FIGURE 1 Valorization strategy from lignin fast-pyrolysis bio-oil obtained by the Lignin Biorefinery Approach (de Wild et al., 2014)

et al., 2013; Vispute, Zhang, Sanna, Xiao, & Huber, 2010; Vithanage et al., 2017). To date, the resulting aqueous phase only generates a limited value, for example, as a source of biogas via anaerobic digestion/wastewater treatment of the low molecular weight substances present such as acetic acid and methanol. The presence of catechol, phenol, *o*-, *p*-, and *m*-cresol, and guaiacol in the aqueous phase at elevated concentrations causes a double economic burden. In addition to the unexploited income, they represent waste that needs to be processed (Black et al., 2016).

cis, *cis*-Muconic acid (MA) is a chemical of recognized industrial value. Primarily it serves as a precursor for the synthesis of adipic acid by hydrogenation (Salvachúa et al., 2018; Vardon et al., 2016). MA can also be converted into caprolactam and terephthalic acid (Pleissner et al., 2019). Adipic acid is a drop-in product for the petrochemical industry with an average market price of 1,650 euro/ton and a turnover of 3,737.8 kilotons in 2016 (Niu, Draths, & Frost, 2002; Coherent Market Insights, Seattle, WA). From 2004 to 2014, the market price of adipic acid fluctuated strongly, varying between 750 and 2,500 euro/ton (Tecnon Orbichem, UK). Petrochemical production of adipic acid is associated with a high-energy demand and CO_2-eq emission (J. B. van Duuren et al., 2011). Recently, a life cycle assessment (LCA) has been performed for the bio-based production of adipic acid from lignin-derivatives aromatic compounds (Corona et al., 2018). The crucial lignin depolymerization step was achieved by an alkaline catalyzed process, yielding a high heterogeneous mixture of aromatics. Overall, the bio-based adipic acid process was predicted to lead to a reduction of 4.870 kg CO_2-eq/ton (~79%) as compared with the conventional chemical process. From this study, it is clear that novel depolymerization processes of lignin must be developed to obtain more defined aromatic mixtures, which eventually will be less toxic for the biocatalyst during bioconversion. In an initial fed-batch process with pulse-wise feeding, Kohlstedt et al. (2018) demonstrated the metabolic conversion of the defined monomeric aromatics catechol, phenol, and cresols from lignin after hydrothermal treatment besides glucose as carbon (C) substrate. This study describes an integrated biotechnological and chemical process comprising fast-pyrolysis of lignin in which the same aromatics are bioconverted to produce MA with the robust and versatile soil bacterium *Pseudomonas putida* (Jiménez, Miñambres, García, & Díaz, 2002; Nelson et al., 2002; Nikel, Martínez-García, & de Lorenzo, 2014; Shingler & Moore, 1994; Vardon et al., 2015). The usage of small aromatics present in the aqueous phase of lignin for MA biosynthesis was simulated. The LIBRA fast-pyrolysis process in combination with hydrodeoxygenation processed softwood lignin leading to the absence and a low concentration of syringol and guaiacol, respectively. We show that the resulting catechol can yield 95 mol% of MA using engineered *P. putida* strains. We also developed a downstream process for MA purification at large scale. From the actual values obtained from the bioprocessing with small aromatics as a model compound, we carry out an LCA and cost analysis, which predicts a less severe environmental impact compared with the petrochemical-based process.

2 | MATERIALS AND METHODS

2.1 | Lignin feedstocks

With regard to the most important botanical families of lignocellulosic biomass, various lignin types were considered. Two softwood kraft lignins, Lignoboost™ (KL1-SW; spruce; 2.1 wt% sulfur; Innventia, Sweden) and IndulinAT™ (KL2-SW; pine; 1.3 wt% sulfur; MeadWestvaco Corporation), and a soda lignin from mixed wheat straw/Sarkanda grass, named Protobind™ 1000 (SL-W; ALM Industries Ltd., India), were obtained commercially (Beis et al., 2010). Both kraft lignin types contain covalently bonded sulfur. Lignoboost™ contains more sulfur, as pulp mills in Sweden generally use higher sulfur levels than in North America. In addition, three organosolv lignins were extracted from spruce (OS1-SW), poplar (OS2-HW), and wheat straw (OS3-W) using an acid-catalyzed ethanol-based organosolv process (Wildschut, Smit, Reith, & Huijgen, 2013). The three organosolv lignins were prepared in-house by treatment with a 60 wt% ethanol–water solution at 200°C for 30 min.

2.2 | LIBRA lignin pyrolysis

KL1-SW, KL2-SW, OS1-SW, and SL1-W were pyrolyzed via the LIBRA process. Batch experiments were conducted at atmospheric pressure, the feeding rate was 500 g/hr, and approximately 1 kg of hot sand was fluidized at five times the minimum fluidization velocity of 20 cm/s. Fast-pyrolysis in the presence of nitrogen was applied to 140 g of KL1.1-SW, OS1.1-SW, and SL1.1-W at 450°C and to 450 g of KL1.2-SW and KL2.1-SW at 550°C. The latter pyrolysis setup was repeated for KL1.3-SW under direct hydrodeoxygenation conditions by adding hydrogen and nitrogen in the ratio of 10% and 90% vol/vol, respectively. After passing through a cyclone to separate the char, pyrolysis vapors were collected in two successive 250 ml ice/water-cooled impingers (Figure S1) and downstream in an electrostatic precipitator (ESP) at room temperature. In the impingers, a two-phase liquid product with an upper aqueous layer and a bottom organic layer was obtained. The phases could be separated by gentle decantation. The aqueous phase from the two impingers, which contained substantial concentrations of catechol, was used for further biochemical processing. Organic degradation products in aerosol form were not captured in the impingers but were precipitated/condensed in the ESP. The composition of the bio-oil from the ESP is similar to the decanted organic phase of lignin bio-oil separated from the aqueous phase (de Wild et al., 2017). Table 1 lists the volumes of the organic and aqueous phases. The organic fraction obtained in the impingers was not taken into account. The concentrations of the monomeric compounds in both phases were used for simulation (Tables 1 and S1). Finally, low-boiling organic substances such as methanol, acetone, and acetic acid were trapped at −30°C in a desublimator downstream of the ESP. The noncondensable pyrolysis gas fraction was finally flared in a recovery boiler. The catechol and partial phenol and cresols in the aqueous phase of the lignin pyrolysis

TABLE 1 The LIBRA fast-pyrolysis process and the obtained concentrations of the defined monomeric aromatics in the aqueous phase of lignin bio-oil

Lignin type	Amount of lignin (g)	Pyrolysis (°C)	Fluidization gas (wt%)	Organic phase (g)[a]	Aqueous phase (g)	Concentration (mM) (before/after steam bath distillation)[b]				
						Catechol	Phenol	Guaiacol	*m,p*-Cresol	*o*-Cresol
KL1.1-SW	140	450	N_2 (100)	22.4	13.0	78.20/5.69	12.53/0.00	8.81/0.12	2.84/0.04	7.90/0.01
KL1.2-SW	450	550	N_2 (100)	82.5	87.1	185.31/5.90	68.09/1.98	1.97/0.02	2.53/0.04	23.69/0.78
KL1.3-SW	450	550	N_2/H_2 (90/10)	69.2	83.2	183.54/6.92	112.63/3.36	1.32/0.00	9.97/0.07	24.27/0.66
KL2.1-SW	450	550	N_2 (100)	76.1	96.4	116.85/6.30	41.04/0.03	14.33/0.00	6.35/0.02	13.01/0.07
OS1.1-SW	140	450	N_2 (100)	26.6	25.2	176.01/5.57	102.10/2.21	4.80/0.28	2.84/0.03	8.83/0.25
SL1.1-W	140	450	N_2 (100)	23.8	29.4	44.87/4.82	22.82/2.24	5.29/0.95	4.92/0.11	2.19/0.16

Abbreviations: HPLC, high-performance liquid chromatography; LIBRA, lignin biorefinery approach.
[a]The organic phase in the electrostatic precipitator during the LIBRA process.
[b]The defined aromatics present in the aqueous phase of the bio-oil measured by HPLC.

bio-oil were purified by steam bath distillation. To 1 ml of the solution, 50 ml of Milli-Q water was added. The resulting solution was heated at 120°C for 2 hr in a 500 ml round-bottom flask with boiling chips. Steam was generated by boiling water in a separate 5 L flask, and the distillate was cooled in a separate 1 L flask. The residue of the pyrolysis bio-oil from the 500 ml flasks was transferred to a bottle, flushed with argon and stored at −20°C.

2.3 | Strains and culture conditions and molecular biology experiments

The bacterial strains used in this study are listed in Table S2. Unless otherwise stated, bacteria were grown in Luria broth (LB; 10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl dissolved in water and autoclaved). Batch cultures were performed in Erlenmeyer flasks shaken at 200 rpm. *Escherichia coli* and *P. putida* were cultured at 37°C and 30°C, respectively. The following four stock solutions dissolved in water were prepared separately and autoclaved: a 10X stock solution of M9 salts prepared with 42.5 g $Na_2HPO_4{\cdot}2H_2O$, 15 g KH_2PO_4, 2.5 g NaCl, and 5 g NH_4Cl per 500 ml; 120.37 g/L $MgSO_4$; 200 g/L citrate; and 16 g/L agar. The components of each solution were diluted in sterile water to a final concentration of 1X M9 salts, 0.24 g/L $MgSO_4$, 2 g/L citrate and, where required, 14 g/L agar. If necessary, additional antibiotics were added. From ampicillin (Amp) 150 mg/mL stock solution in water at −20°C, solutions were prepared at a concentration of 100 μg/ml for *E. coli* cells and 500 μg/ml for *P. putida* cells. From kanamycin (Km) 50 mg/ml stock solution in water at −20°C, a solution at a concentration of 50 μg/ml was prepared. The following supplements were added as required: 80 μg/ml 5-bromo-4-chloro-3-indolyl-β-*D*-galactopyranoside (X-gal) prepared from a 400 g/L stock solution in dimethylformamide at −20°C, 0.24 g/L isopropyl-β-*D*-1-thiogalactopyranoside (IPTG) prepared from a 238.30 g/L stock solution in water at −20°C, and 15 mM 3-methyl-benzoate (3MB) prepared from a 68.075 g/L filter-sterilized stock solution in water. An electroporation buffer consisting of an autoclaved solution of 102.69 g/L sucrose in water was stored at room temperature. The culture of *P. putida* or *E. coli* cells was monitored during growth by optical density at 600 nm (OD_{600}) using a UV-1600PC spectrophotometer (Radnor, PA).

The pEMG plasmid was used to generate directed scarless deletion and integration of sequences in the genome of *P. putida* KT2440 as described by Martínez-Garcia and de Lorenzo (2011; Table S3). This method is based on the homologous recombination, which causes double-strand breaks in the genome of *P. putida* followed by in vivo I-SceI cleavage. Transient expression of the nuclease is controlled by Pm in pSW-I, which is induced in the presence of 3MB. The deletion of *catB/catC* (KT2440-JD2S) and the integration of Pem7, a constitutive promoter, upstream of *catA* in *P. putida* (KT2440-BN6) were performed consecutively. For the genetic modifications used to obtain *P. putida* KT2440-JD2S and KT2440-BN6, the upstream (TS1) and downstream (TS2) regions flanking the region to be deleted and inserted, respectively, and the insertion Pem7 were amplified separately using Phusion High-Fidelity polymerase (Thermo Fisher Scientific, Germany). The following primers were used for ΔcatB/C: TS1_catB/C-F/TS1_catB/C-R and TS2_catB/C-F/TS2_catB/C-R and for the integration of Pem7: TS1_Pem7-F/TS1_Pem7-R and TS2_Pem7-F/TS2_Pem7-R; and Pem7-F/Pem7-R (Table S4). First, the pEMG plasmid was linearized with the endonuclease *Sma*I. For the Δ*catB/catC*, the products (TS1 and TS2) were fused and ligated via Gibson assembly into the restriction site *Sma*I. Similarly, for the insertion of Pem7 upstream of *catA*, TS1, TS2, and the promoter to be inserted (Pem7) were fused and ligated. Both of the two resulting plasmids, pEMG-Δ*catB/catC* and pEMG-Pem7, were transformed separately into *E. coli* DH5αλpir via electroporation, and the culture was plated on LB-Km plates supplemented with X-gal and IPTG to identify potential positive clones through visual screening based on blue and white color differentiation of colonies. Putative positive clones were tested for the presence of the TS1/TS2 insertions by colony polymerase chain reaction (PCR) using pEMG-F/pEMG-R and confirmed by sequencing the corresponding plasmids. The pEMG derivates were isolated with the Miniprep Kit (Qiagen, The Netherlands) of *E. coli* DH5αλpir and transformed into *E. coli* CC118λpir. The latter strain was used to deliver

the plasmid in *P. putida* KT2440 via conjugation-based triparental mating assisted by *E. coli* HB101 (pRK600) as described in de Lorenzo and Timmis (1994) and Martínez-Garcia and de Lorenzo (2011). The bacterial mixtures were resuspended in 10 mM $MgSO_4$, and appropriate dilutions of the pellet were plated onto M9 citrate (as a selective C substrate for *Pseudomonas*) plus Km. Since pEMG-derived plasmids cannot multiply in *P. putida*, Km^R clones can grow only by the cointegration of the construct in the genome of the recipient strain. Delivery of the pSW-I plasmid into competent *P. putida* was performed by electroporation of Km-resistant cells and 50 ng of the plasmid. Sequentially, the cells were plated on LB-Km+Amp. The sucrose solution was used during the electroporation as a wash/electroporation buffer. The presence of the plasmid was confirmed by PCR using pSW-F/pSW-R. The induction of the I-SceI enzyme in co-integrated clones harboring the pSW-I plasmid was initiated by adding 3MB to 5 ml of LB-Amp medium. The culture was incubated at 30°C for 14 hr and plated on LB-Amp plates. Loss of the cointegrated plasmid was evaluated by selecting Km-sensitive clones on LB-Km plates. The deletion of *catB/catC* in *P. putida* KT2440-JD2S and insertion of Pem7 in KT2440-BN6 were confirmed by PCR using Check-F/Check-R and TS1-Pem7-F/TS2-Pem7-R, respectively. The curation of pSW-I was achieved by several passages in LB without antibiotics.

2.4 | Biological conversion of catechol from lignin into MA

From a frozen stock at −80°C, *P. putida* BN6 was plated on E-2 mineral medium with 10 mM glucose (Hartmans, Smits, van der Werf, Volkering, & de Bont, 1989). A batch culture of 50 ml with the same medium containing 5 g/L glucose was inoculated from the plate and grown for 12 hr, from which a second batch culture was inoculated with the same medium at an OD_{600} of 0.2. From the latter batch culture, the cells were harvested in the exponential growth phase. Next, the cells were rediluted to an OD_{600} of 0.2 in the same medium supplemented with 1.5 mM purified or nonpurified catechol by steam bath distillation from the aqueous phase of lignin bio-oil. Each supplement was assayed three times in 1 ml culture volume in 48-well flower plates in a BioLector (m2p-laps, Germany). The cultivation lasted 24 hr at a relative humidity of 85% at 1,300 rpm.

2.5 | Upscale biological production of MA from benzoic acid

From a frozen stock at −80°C, a 5 ml LB batch culture was inoculated with *P. putida* KT2440-JD2S and grown for 22 hr. With 1.33 ml of the LB batch culture, a 60 ml batch culture of E-2 mineral medium with 10 mM glucose was inoculated and grown for 26 hr. With 14 ml of the latter batch culture, a 660 ml batch culture was inoculated with the same medium supplemented with 1 mM benzoate and grown for 23 hr. The latter culture was used as a preculture for the fermentation. The Erlenmeyer flasks of the batch cultures were shaken at 100 rpm. In a 72 L bioreactor (Braun Biostat UD, Germany), a pH-stat fed-batch culture with an initial working volume of 30 L was carried out with the same initial medium as the preculture without the addition of benzoate. After the inoculation, the measured oxygen saturation was maintained at 50% in the culture by sparging with air at a rate of 15–25 L/min and an adjusted stirring speed. The pH was regulated to at least pH 7 with 20 wt% NaOH during the batch culture phase. After 16 hr, the fed-batch culture phase was initiated by adding 1 mM benzoate and by starting the exponential feeding with a solution containing E-2 mineral medium, 1.4 M glucose and 72 g/L ammonium sulfate at an installed biomass growth rate (μ) of 0.04 hr^{-1}. Since the maintenance factor increases during the process and to ensure steady growth, the progressive maintenance was set to 2% hr^{-1}, starting at 0.037 $g_{glc} \cdot g_{DCW}^{-1} \cdot hr^{-1}$ (van Duuren et al., 2013). During the pH-stat process, the addition of benzoate was coupled to the pH-regulation at pH 7 by titrating with a solution containing E-2 mineral medium, 1.2 M benzoic acid and 2.4 M sodium hydroxide (van Duuren et al., 2012). On the basis of the absolute number of bacteria, a μ of 0.041 hr^{-1} (r^2 = .94) was obtained. The number of living cells was steady during the fed-batch culture phase. After 78 hr, the addition of benzoate was stopped. During the final 9 hr of the fermentation, the pH was again regulated at pH 7 with 20 wt% NaOH to avoid the presence of residual benzoate in the product. To reduce the resource demands, the feeding rate of glucose was adjusted to a linear rate of 21.70 ml/hr, which corresponds to 37.4% of the last glucose inflow. On the basis of the foam level measurement in the reactor, 85 ml of Antifoam 204 (Sigma-Aldrich, St. Louis, MO) was added to the medium. With the described process, a 42.5 L broth with 1695 g of MA was obtained after 87 hr. The cells were separated from the medium by microfiltration. This process step was performed by a UFI-TEC cross-flow microfiltration system (UFI-TEC, Germany) with 4*TAMI 0.2 µm membranes (TAMI Industries, France) at 1.5 bar, 15°C and a duration of 1.75 hr. The biomass was concentrated in 4.3 L of supernatant containing 243.8 g of MA, which was discarded. Next, 2.1 L of 32 wt% HCl was added to 38.2 L of medium. After adjusting the pH to 2.7, which is below the second pKa value of MA, the compound became very insoluble in water (Table S5). Accordingly, 1,450.4 g of MA could be acquired by overnight precipitation. The resulting solution (22 L) in which 1.21 g of MA remained was decanted. The solid MA was washed sequentially with 45 and 20 L of water. In addition to impurities, the 45 and 21 L of discarded water used for washing contained 16.61 and 8.45 g of dissolved MA, respectively. From the remaining 17.1 L of MA suspension, the compound was spray-dried with 50°C air on a surface at 120°C for 9.38 hr. For this last process step, an absolute yield of 0.80 wt/wt was obtained, affording 1,153 g of MA as a white powder, the purity of which was determined (Table S5).

2.6 | Sample analysis

The concentrations of catechol, phenol, guaiacol, and *o*-, *m*-, and *p*-cresol in the aqueous phase of lignin bio-oil were analyzed via ultra-high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA). The flow rates of Eluent A (0.025 wt% H_3PO_4 in water) and B (acetonitrile) were 1 ml/min, and the oven temperature was adjusted to 25°C. A Merck Purospher STAR RP-18 column (Merck Millipore, Burlington, MA) and a G4212A DA detector (Agilent Technologies) were

used for separation and detection, respectively. The aromatic compounds were quantified at 210 nm, and MA was quantified at 260 nm. Aliquots of the organic phase of the LIBRA process were analyzed with gas chromatography coupled with mass spectrometry (GC–MS) as described by de Wild, den Uil, Reith, Kiel, and Heeres (2009). During the upscale pH-stat fed-batch culture, the total number of cells was determined using a THOMA cell chamber (Glaswarenfabrik Karl Hecht GmbH & Co. KG, Germany). The number of living cells was determined as the colony-forming units counted on a plate of nutrient agar (Merck, Germany) after 24 hr at 30°C. Furthermore, as described by Neu et al. (2016), the following analytes were measured from the fermentation samples: acids (g/L), sugars (g/L), cations and anions (mg/L), ammonium nitrogen (NH_4^+–N; mg/L), and Kjeldahl-nitrogen (N_{kjel}; mg/L). Dry matter ($DM_{105°C}$; wt%), organic dry matter (oDM; wt%) and conductivity (mS/cm) were analyzed as described by Wirth and Mumme (2013). Further information can be found in Table S5.

2.7 | Description of the simulated scenarios for the calculation

To determine the impact of different process step settings to produce adipic acid with the proposed integrated biotechnological and chemical process, Scenarios A–E were performed from the Lignoboost™ pyrolysis sample KL1.2-SW and Scenario F from the Lignoboost™ pyrolysis sample KL1.3-SW. The greenhouse gas emissions (CO_2-eq/ton), energy demand (kWh/ton) and variable costs (euro/ton) were determined. The optimization potential is maximum for the rectification and fermentation process steps, which cause the highest variable costs. Therefore, a sensitivity analysis was performed on both process steps. This was made possible, respectively, by plotting variable costs against the produced mass of adipic acid (g) per liter of the aqueous phase of the bio-oil (Figure S4a) and against a variable titer of MA (Figure S4b). In Scenarios A and C, among the aromatics in the aqueous phase, only catechol is metabolically converted into MA. In Scenarios B, D, E, and F the resulting aromatic mixture of catechol, phenol, guaiacol, and *o*-, *p*-, and *m*-cresol is metabolically converted. In Scenarios A and B, and C–F, a titer of 39.9 and 62.5 g/L MA, respectively, was applied. In Scenarios A–D, the direct use of the resulting aromatics in the aqueous phase was simulated. The concentration of catechol in the organic phase of lignin bio-oil can be expected to be decreased by controlled cooling of pyrolysis vapors (Chang, Wu, Lin, Wan, & Lee, 2012). It is expected that the oligomeric phenolic compounds first condense due to their higher boiling points and that catechol and other monomeric compounds condense sequentially with the water phase. In Scenarios E and F, this approach was simulated. Catechol can dissolve in water up to 451 g/L. To simulate the latter approach, the measured concentrations of catechol, phenol, guaiacol, and *o*-, *m*-, and *p*-cresol in the aqueous and organic phases were combined (Tables 1 and S1). To calculate concentrations the volume of the aqueous phase and the mass of the monomeric compounds in the organic phase were taken into account. The effect of hydrodeoxygenation can be defined as sample KL1.3-SW was pyrolyzed in the presence of hydrogen. CHEMCAD 7 (Chemstations) was used to calculate the internal process energy demand of the experimental setup. Figure 2 shows the system unit operations and the streams. Table S6 shows for Scenarios A–F the simulated concentrations of the defined monomeric aromatics before and after rectification. In addition, the processing conditions of the integrated biotechnological and chemical process is described. For all energy inputs, an efficiency of 96% was considered. For electricity, heating and cooling, the following market prices and CO_2-eq emissions were used respectively: 0.115, 0.03, and 0.0046 euro/kWh and 0.527, 0.203, and 0.0209 kg CO_2/kWh (Eurostat, Luxembourg; Pfafflin & Ziegler, 2015; Carbon Independent, UK). The energy demand and CO_2-eq emission of the fast-pyrolysis process was not taken into account. The pyrolysis process is considered an endothermic process with lignin as a resource at a temperature >500°C, as described by Yang, Yan, Chen, Ho Lee, and Zheng (2007). Furthermore, the process, which is the addition of hydrogen and the controlled cooling of pyrolysis vapors, is primarily carried out for the accumulation of phenol substitutes in the organic oligomeric phase of lignin bio-oil to produce phenol–formaldehyde resins.

2.8 | Limited LCA goal, scope, and settings

The following cumulative energy demand (CED) and emissions for the petrochemical production of adipic acid were taken into account: 28,954.75 kWh/ton, 5,070 kg CO_2/ton, and 60.2 kg N_2O/ton (J. B. van Duuren et al., 2011). An average reduction of 80% N_2O emission by catalytic decomposition was considered. The greenhouse effect with N_2O is 298 times stronger than that with CO_2 (global warming potential in 100 years) according to the Intergovernmental Panel on Climate Change (IPCC, Switzerland) 2007 (Solomon et al., 2007). The goal and scope of the limited LCA were determined in a similar way to J. B. van Duuren et al. (2011). The CEDs for the bioreactor feedstocks for bacterial growth described by the same authors were used (Table S12). The correlated CO_2-eq emissions were determined using the heat value of natural gas (Table S10). For ammonium sulfate, CED and CO_2-eq emissions were applied in accordance with the production and nutrient values defined by Fertilizers Europe, Belgium. The reported NaOH and HCl values were adopted from J. B. van Duuren et al. (2011). The values for glucose (CED of 1,069.45 kWh/ton; 280.20 kg CO_2/ton; 9.64 kg N_2O/ton) and for aromatics from lignin (CED of 1,158.34 kWh/ton; 403.10 kg CO_2/ton) were determined. For glucose production, the account was taken of the agricultural value chain for beet sucrose production and its sequential sugar production (Brehmer, Boom, & Sanders, 2009; Brehmer, Struik & Sanders, 2008; Jensen & Morin, 2015). The CO_2-eq emission was calculated using the combined heat values of: Natural gas (0.203 kg CO_2/kWh) for fertilizers and pesticides; oil (0.245 kg CO_2/kWh) for irrigation, farming, transportation and storage handling, and electricity consumption (0.527 kg CO_2/kWh) for drying. In addition, the emissions caused by production, use and nutrient content of NPK 15-15-15 were considered as defined by Fertilizers Europe. For lignin, the energy demands and CO_2-eq emissions of farming, sizing, transportation, and storage were taken into account accordingly. In addition, the energy

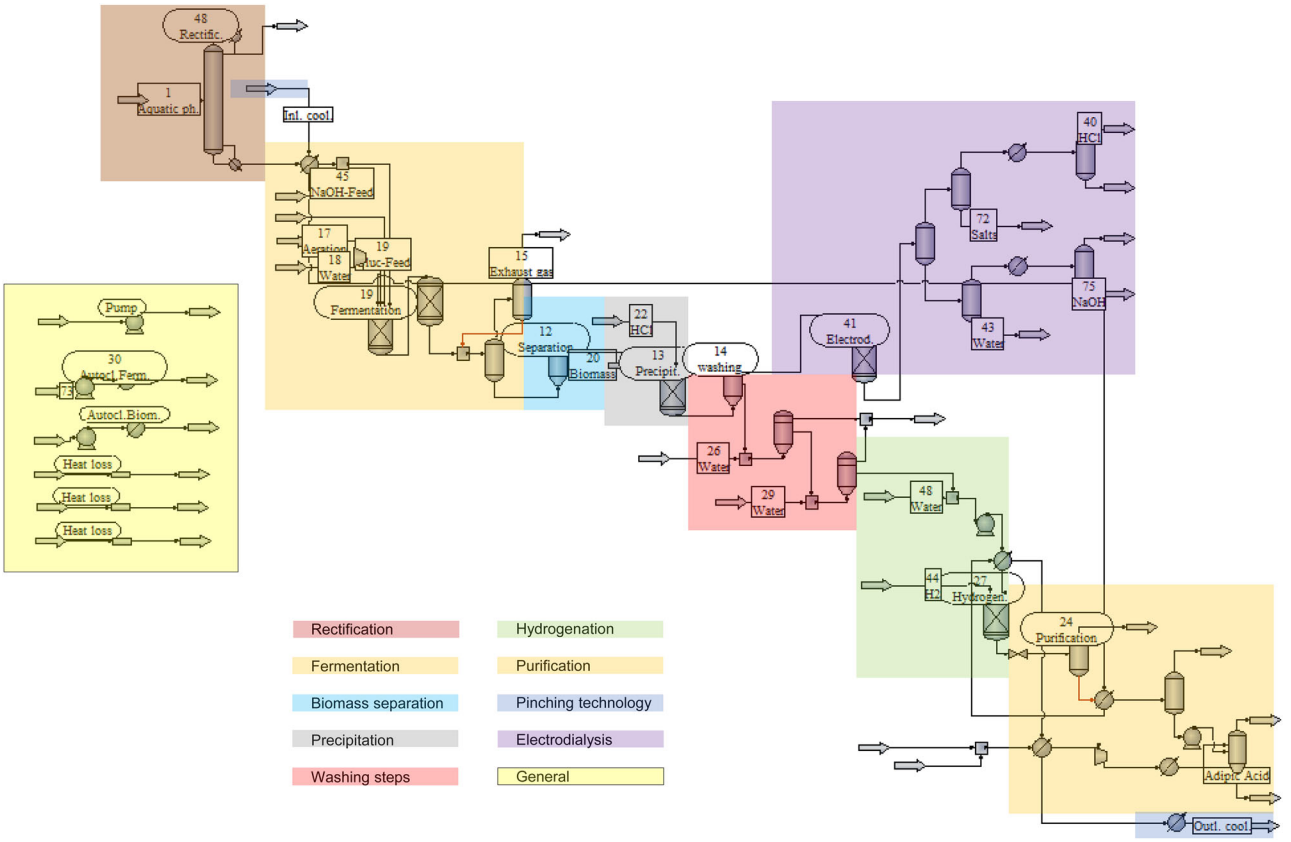

FIGURE 2 Flowchart of the CHEMCAD 7 simulated integrated biotechnological and chemical process for the production of adipic acid from the defined aromatics present in the aqueous phase of lignin bio-oil [Color figure can be viewed at wileyonlinelibrary.com]

demands for H_2SO_4 and NaOH for the production of Lignoboost™ were covered. The energy demand for the LignoBoost Process (31.5 GJ/ton) is covered by the pulp mill (Lettner et al., 2018). The amount of lignin, which represents an excess of energy that can be removed from a mill, is more than 25% of the total and depends on the combustibility of the black liquor and the operability of the recovery boiler (Valmet, Finland). CO_2 is used for the production of lignin, however, it was not taken into consideration because it is collected from in-house lime kiln flue gases. For the two described chemicals, the heat value of natural gas was used to calculate the associated CO_2-eq emission. The values for lignin were multiplied by the absolute yield from the two-phase bio-oil. Also, the use of char as organic fertilizer at 35 wt/wt was considered. For hydrogen and the recycled NaOH and HCl using bipolar membrane electrodialysis, the following values were taken into account: CED of 32,900 kWh/ton, 17,338.3 kg CO_2/ton, and 0 kg N_2O/ton; CED of 2,440 kWh/ton, 1,285.88 kg CO_2/ton; and 0 kg N_2O/ton; and CED of 2,680 kWh/ton, 1,412.36 kg CO_2/ton; 0 kg N_2O/ton, respectively, at a market price of 2,047.00, 281.03, and 310.00 euro/ton (Davis, Chen, Baygents, & Farrell, 2015; Hydrogen Energy Systems, Akron, OH). For recycled NaOH and HCl, the parameter settings were the same as described in Davis et al. A utilization of electrodialysis at 70% was considered, which results in total costs equivalent to 1.7 times the energy costs of producing both compounds at an electricity market price of 0.07$/kWh. For NaOH and HCl, energy costs were defined between 1.75 and 6.3$/kmol. Total costs include all peripherals, maintenance, capital costs, and membrane replacement every 2 years. On the basis of this information, the following calculations were made: NaOH (25 kmol/ton) × (((1.75$/kmol + 6.3$/kmol) × 2^{-1}) × $0.07^{-1}$$/kWh) × 1.7 × 0.115 euro/kWh = 281.03 euro/ton; HCl (27.43 kmol/ton) × (1.75$/kmol + 6.3$/kmol) × 2^{-1}) × $0.07^{-1}$$/kWh) × 1.7 × 0.115 euro/kWh = 308.32 euro/ton. The concentration obtained based on the feed solution was assumed to be 77 wt%. For water, an integral market price of 3.47 euro/ton, a CED of 1.06 kWh, and emission of 1.38 kg/ton CO_2 were used (OECD, Germany; Stowa, the Netherlands).

3 | RESULTS AND DISCUSSION

3.1 | Characterization of lignin types

Phenol–formaldehyde resins are prepared from the organic phase of lignin fast-pyrolysis bio-oil as a substitute for phenol. We quantified the concentrations of syringol, guaiacol and catechol, as their presence has a negative effect on the resin properties. Five lignin types (OS1-SW, OS2-HW, OS3-W, KL2-SW, and SL-W), each with a mass of 0.5 mg, were pyrolyzed at 500°C. Based on the analysis of the organic phase and

aqueous phase with GC–MS (Table S7) catechol was present at a high abundance in both phases. To realize a high added value utilization of this compound using an integrated biotechnological and chemical process, we selected catechol as a target substrate for metabolic conversion into MA. Hydrogenation of the acid affords the bulk chemical adipic acid (Niu et al., 2002), which is mainly used in the industry for the production of resins. Catechol metabolizes efficiently at a high rate into MA in one enzymatic step (van Duuren, Wijte, et al., 2011; Jiménez, Pérez-Pantoja, Chavarría, Díaz, & de Lorenzo, 2014). Since catechol is a secondary thermal conversion product of guaiacol, the presence of both compounds after pyrolysis is a strong indication of the potential of the particular lignin as a resource for this metabolic conversion (Ben et al., 2013). Softwood lignin gave the highest relative yields of guaiacol analogs and catechol without syringol. This result is consistent with the fact that coniferous lignin consists almost entirely of linked guaiacyl moieties, in contrast to deciduous and herbaceous lignins. Therefore, we selected softwood lignins as the most promising type of lignin for the proposed integrated biotechnological and chemical process. Moreover, the absence of syringol promotes the use of the phenolics and alkylated phenolics in the organic phase of lignin bio-oil for the production of phenol–formaldehyde resins.

3.2 | Fast-pyrolysis

With the LIBRA pyrolysis process, four lignins (KL1-SW, KL2-SW, OS1-SW, and SL1-W) were pyrolyzed (Table 1). The monomeric aromatics phenol, guaiacol, and *o*-, *m*-, and *p*-cresol accumulated at an elevated concentration in the aqueous phase of lignin bio-oil in addition to catechol. After the steam bath distillation of this phase, the strain *P. putida* KT2440-BN6 could convert catechol from all four lignin types into MA (Figure S2). Therefore, a widespread application of this integrated biotechnological and chemical process is expected. The physiological response is defined by the yield, μ_{max}, lag phase, exponential growth phase, and final OD_{600}. In the presence of catechol derived from KL1.2-SW, KL1.3-SW, KL2.1-SW, and OS1.1-SW after steam bath distillation, similar values are shown in as in the presence of synthetic catechol besides glucose as C substrate. We achieved a percent yield of up to 95 mol%. The strain was unexpectedly able to convert catechol from KL2.1-SW from IndulinAT™ without steam bath distillation. To understand this result in more detail, we analyzed the aqueous phases from KL1.2-SW from Lignoboost™ and KL2.1-SW from IndulinAT™ by GC–MS. The following substances were only present in the aqueous phase from KL1.2-SW: methyl acetate, hydroxyacetaldehyde, furfural, 2(5H)-furanone, 5-hydroxymethylfurfural (5-HMF), 4-hydroxybenzaldehyde, 4-hydroxyacetophenone, resorcinol, and levoglucosan (Table S8). Levoglucosan and furfural represent the strongest indicators that most of these compounds are thermochemically degraded carbohydrates (Greenhalf, Nowakowski, Harms, Titiloye, & Bridgwater, 2012; Jiang et al., 2016). In particular, the characterization of furfural and 5-HMF as fermentation inhibitors, explains the hindered growth in the presence of the aqueous phase of lignin bio-oil from KL1.2-SW (Jiang et al., 2016). To avoid the presence of undesired pyrolysis products, the process can best be implemented with pure lignin. Pyrolysis of KL1.3-SW from Lignoboost™ in the presence of additional hydrogen to implement hydrodeoxygenation increased the thermochemical conversion of guaiacol into lignin bio-oil. As described by Ben et al. (2013), guaiacol was converted into catechol and phenol, which was underlined by the increased concentration of both compounds (Tables 1 and S1). Since guaiacol hampers the full use of the organic oligomeric phase of lignin bio-oil, this specific conversion promotes the substitution of phenol for the production of phenol–formaldehyde resins.

3.3 | Value chain

We performed a limited LCA analysis including variable costs for the development of an integrated biotechnological and chemical process. We used a combination of upstream and downstream data from the upscale process with the strain *P. putida* JD2S and benzoate as substrate analoge besides glucose as C substrate. Over 87 hr, 39.9 g/L MA was produced in a volume of 42.5 L (Figure S3). It is important to mention that we used benzoate instead of the lignin-derivatives compounds to achieve a high concentration of MA to perform a downstream process, enabling the most realistic scenario for the LCA calculations during purification (Table S5). Total CO_2-eq emission combined N_2O and CO_2 emissions and CHEMCAD 7 listed the resulting energy duties in kWh (Figure 2). For the process and the use of resources, the energy demand, the greenhouse gas emissions, and the variable costs were calculated for the selected simulated scenarios as described in the experimental section (Figures 3, 4, and S9–S14). From the 450 g of Lignoboost™ in samples KL1.2-SW and KL1.3-SW, a total volume of 169.60 and 152.40 ml of lignin bio-oil was achieved, respectively (Table 1). Moreover, the lignin bio-oil consisted of 42.72 wt% (in Scenarios A–E) and 38.71 wt% (in Scenario F) out of the organic oligomeric phase. As part of the small residual aromatics, catechol, phenol, cresols, and guaiacol were measured at an elevated concentration in the aqueous phase, which is valorized with the integrated biotechnological and chemical process. In the bio-oil, the concentration of the residual small aromatic compounds was defined 7.45 and 8.59 wt%, respectively. Concentrated in the aqueous phase, they reach up to 13.02 and 14.02 wt%, respectively. The defined aromatics suitable for metabolic conversion in the aqueous phase were determined: 2.04 wt% for Scenarios A and C, 2.99 wt% for Scenarios B and D, and 9.98 wt% and 12.17 wt% for Scenarios E and F. Obtained absolute yields of adipic acid from the measured defined aromatics in the Scenarios A–F were 0.82, 1.14, 0.83, 1.14, 1.00, and 1.02 wt/wt, respectively. For the CHEMCAD flowsheet, we considered the following system unit operations: rectification, fermentation, biomass separation, precipitation, washing steps, hydrogenation, and purification (Figure 2). Besides, simulating the recycling of sodium hydroxide, hydrogen chloride, and salts via bipolar membrane electrodialysis. Figure 3 shows the influence of the three parameters described for the process units of the integrated biotechnological and chemical process. At a titer of 62.5 g/L MA, the fermentation step in terms of environmental impact and variable costs was optimized for Scenarios C–F. This was the maximum concentration achieved in a laboratory-scale bioreactor experiment with *P. putida* by Kohlstedt et al.

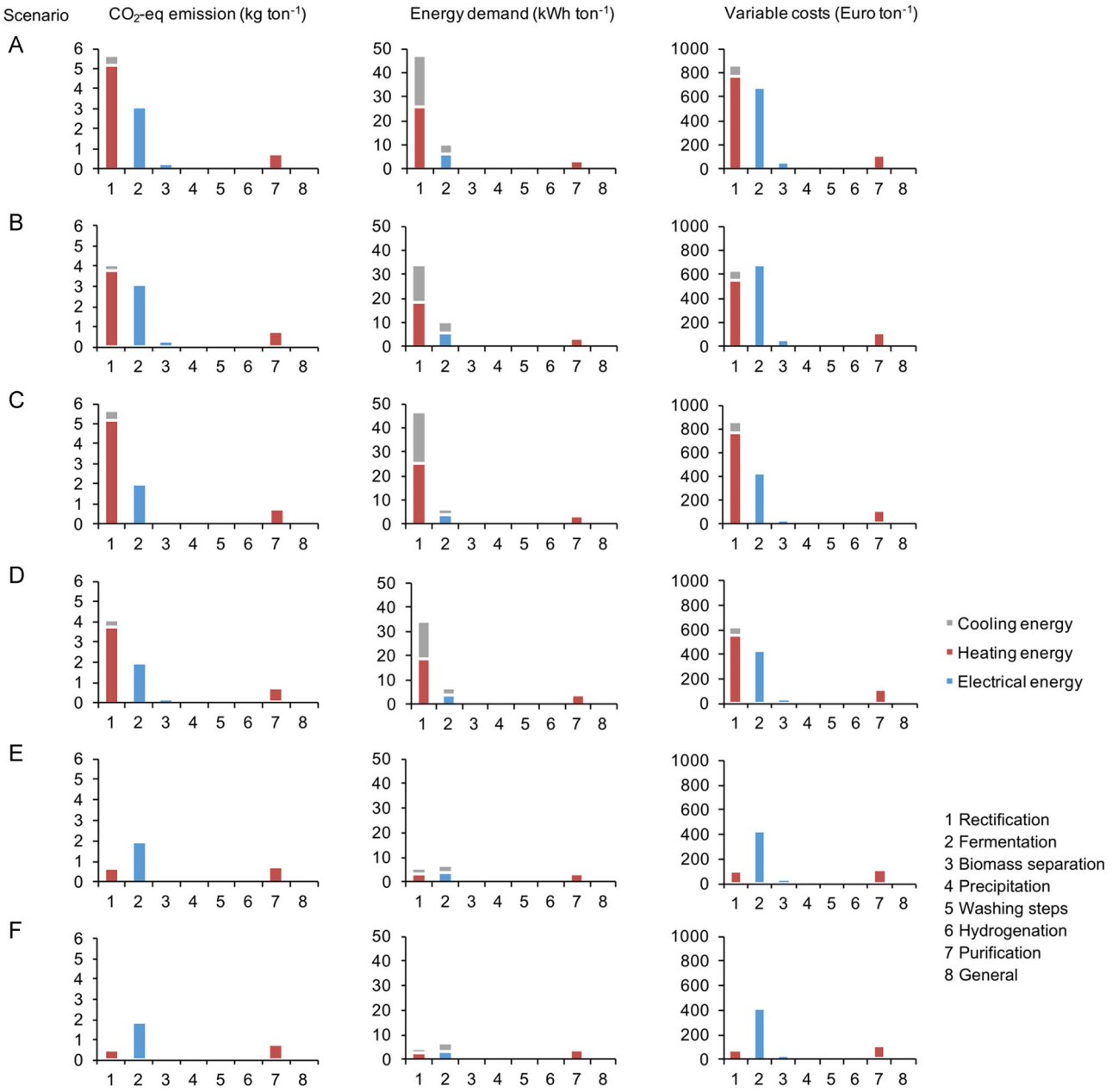

FIGURE 3 Parameter values of process steps of the different scenarios for the integrated biotechnological and chemical process for the production of adipic acid [Color figure can be viewed at wileyonlinelibrary.com]

(2018) with catechol as the substrate. The same feeding strategy for glucose as C substrate applies for these simulations. On this basis, process energies of 4,904.06 and 4,770.34 kWh/ton adipic acid can be saved between Scenarios A and C and B and D, respectively (Figure 3 and Table S16). The fact that catechol is more toxic compared with benzoate related to the logarithmic partition coefficient between *n*-octanol-water ($\log P_{ow}$) of 1.3, renders the process is less robust (Bui, Lau, & Macrae, 2011; Kaneko, Ishii, & Kirimura, 2011; Ramos et al., 2002; Smith & Vinjamoori, 1995). The laboratory-scale bioreactor experiment with catechol as the substrate was scaled up. However, further research is needed to optimize process conditions. Since the fermentation step causes relatively high variable costs the optimization is of high relevance. With 62.5 g/L MA, the efficiency expressed in variable costs approaches its maximum concerning the titer (Figure S4b).

Furthermore, not considered are reduced labor costs as part of the direct costs. Comparing Scenarios A and B indicates that increased metabolic capacity of the strain to convert the defined monomeric aromatics in the aqueous phase leads to a reduction in energy costs due to increased efficiency of the rectification step, from 1,738.56 to 1,504.09 euro/ton adipic acid, respectively (Table S17). As described above, the increased titer also leads to lower energy costs in Scenarios C and D, which we calculated to be 1,457.00 and 1,222.32 euro/ton adipic acid, respectively. In Scenarios E and F, controlled cooling of the pyrolysis gas further increases energy efficiency for the

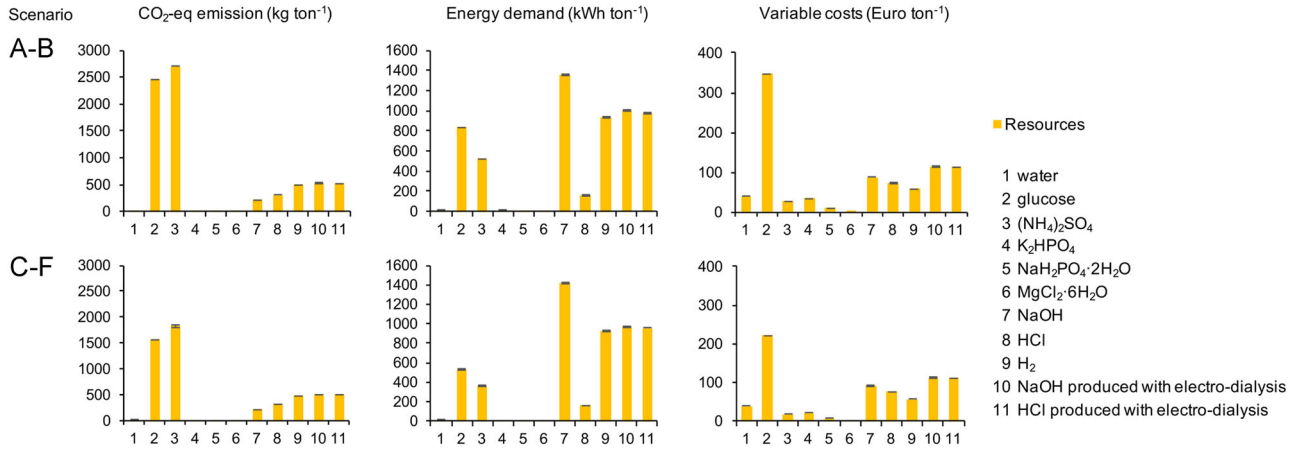

FIGURE 4 Parameter values of resources of the different scenarios for the integrated biotechnological and chemical process for the production of adipic acid [Color figure can be viewed at wileyonlinelibrary.com]

rectification step to a maximum due to a higher concentration of the defined monomeric aromatics in the aqueous phase, resulting in lower energy costs of 701.80 and 660.36 euro/ton adipic acid, respectively (Figure S4a).

3.4 | Prospect

The limited LCA process simulation concludes that the proposed integrated biotechnological and chemical process for adipic acid significantly reduces the greenhouse gas emissions compared with petrochemical production (Figure 5). In addition to the reduced environmental impact, this information is very relevant because of the average cost price of 35.72 euro/ton CO_2-eq emission predicted for 2,022–2,050 (Luckow et al., 2016). Furthermore, resource demands for glucose and ammonium sulfate have a major impact on the emission (Figure 4). Using protein-rich biomass waste streams as a substrate reduces the demand for ammonium sulfate (Pleissner & Venus, 2016). For industrial valorization, we demonstrated based on the simulated Scenario F with hydrodeoxygenation the relevance of the integrated biotechnological and chemical process with (a) a molar mass ratio of catechol, phenol, cresols, and guaiacol of 1.00, 0.34, 0.18, and 0.00; (b) a titer of 62.5 g/L MA; and (c) controlled cooling of pyrolysis vapors to concentrate monomeric aromatics in the aqueous phase (Tables S15–S17). Under these conditions, a contribution margin of 710.30 euro/ton adipic acid can be generated from the defined aromatics in the aqueous phase. For this calculation, we considered a market price of 1,650 euro/ton for adipic acid and the expected savings for the reduced CO_2-eq emission accounted at 35.72 euro/ton. By comparing Scenario F to petrochemical production of adipic acid, the CO_2-eq emission reduces by 58% and the energy demand by 23%. Based on this calculation at a concentration of defined monomeric aromatics of 12.17 wt% with the absolute yield of 1.02 wt/wt, a contribution margin of 88.05 euro/ton can be obtained for the aqueous phase. We did not take into account fixed indirect costs not assigned to the product such as depreciation or administration. To further delineate Scenario F, in Figure 6 a technoeconomic sensitivity analysis is shown in which the contribution margin of the aqueous phase (euro/ton) is based on different market prices for CO_2-eq emission (0–90 euro/ton) and adipic acid (750–2,500 euro/ton). Figure S5 shows the analysis for the other simulated Scenarios (A–E).

The different described scenarios simulated with the limited LCA assume the technical feasibility of a continuous process. Studying the effects of fast-pyrolysis process settings on the concentration of the defined monomeric aromatics and the chemical profile of lignin bio-oil may further increase the impact of the described value chain (Fan et al., 2017). Elucidating the optimal hydrogen demand for reforming monomeric molecules during lignin depolymerization leads up to the more complete conversion of guaiacol analogs in the organic phase by hydrodeoxygenation (Table S1; Azadi et al., 2013). The boiling point of lignin-derived phenolic monomers ranges from 350°C to less than 100°C. Of the defined monomeric aromatics, catechol has the highest boiling point at 245°C. Compared with oligomers, monomers generally have lower boiling points. Catechol and the other defined monomeric aromatics, therefore, separate by controlled cooling of pyrolysis vapors leaving a residue of oligomeric aromatics (Azadi et al., 2013; Chang et al., 2012). To optimize the conditions in the combination of a rectification step further research is required. In addition, metabolic engineering of *P. putida* KT2440-BN6 is necessary to convert phenol and cresols besides catechol into MA, as shown by Nikel et al. (2014), Vardon et al. (2015), Shingler and Moore (1994), Guzik, Hupert-Kocurek, Sitnik, and Wojcieszyńska, (2013), Kalil, Asyigin, and Zaki (2002), and Kohlstedt et al. (2018). The ability to metabolically convert guaiacol by *P. putida* needs to be defined, because it was described for *Amycolatopsis* sp. by Barton et al. (2018). To apply both products in the industry their use must be characterized as a drop-in product. Phenol–formaldehyde resins produced from the organic phase with increased homogeneity are expected to have an appropriate reactivity and reduced viscosity (Vithanage et al., 2017). For adipic acid, which is produced from monomeric aromatics present in the aqueous phase, the presence of methylated adipic acid from *o*-, *m*-, and *p*-cresol must be elucidated (Kohlstedt et al., 2018). Cresols can be poorly separated having a boiling point of 191–203°C while phenol has a boiling point of

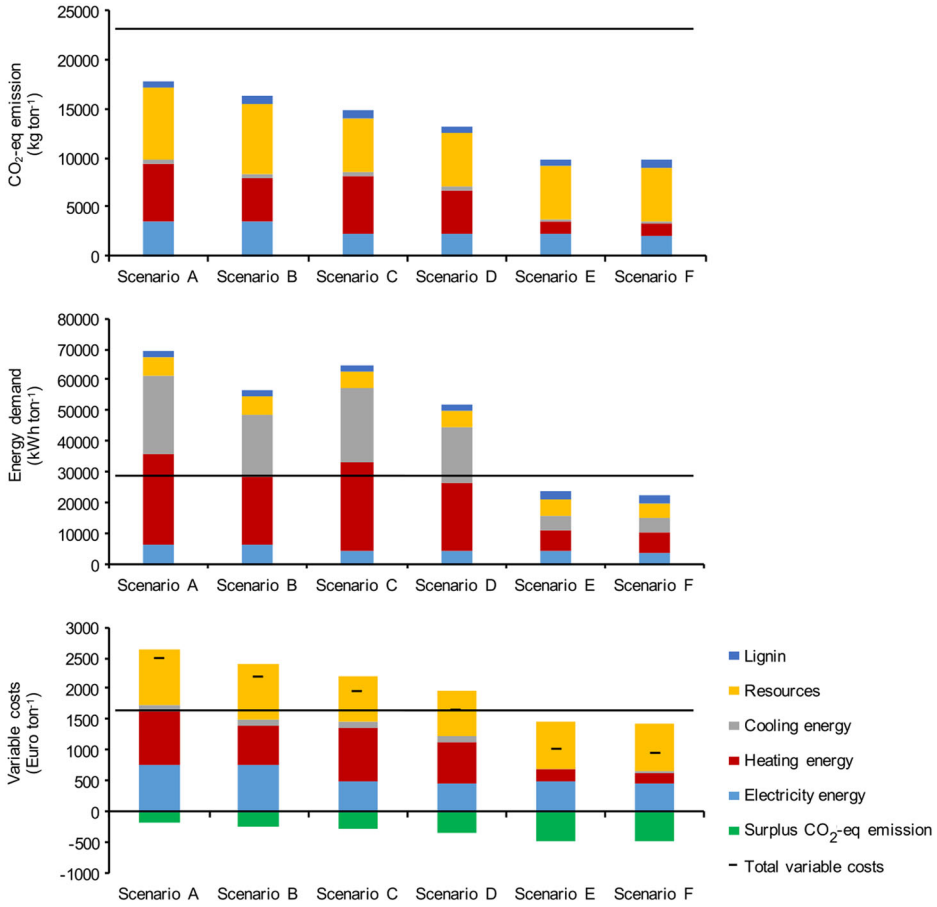

FIGURE 5 Parameter values of the different scenarios for the integrated biotechnological and chemical process to produce adipic acid compared with parameter values of the petrochemical process (black line). The shown data for petrochemical production of adipic acid concerning the CO_2-eq emission and the energy demand come from J. B. van Duuren et al. (2011). Variable costs correspond to the average market price of adipic acid from 2004 to 2014 (Tecnon Orbichem, UK) [Color figure can be viewed at wileyonlinelibrary.com]

182°C. Furthermore, both aromatics are converted by phenol hydroxylase enzymes of the *dmp* encoding operon of the pVI150 catabolic plasmid of *Pseudomonas* sp. Strain CF600 (Shingler & Moore, 1994).

We provided a promising value addition to commercialize the two-phase lignin bio-oil. An absolute yield of bio-oil of 0.38 and 0.34 wt/wt from lignin for Scenario E and F was obtained. The oligomeric organic and the monomeric aqueous phases of the bio-oil of both scenarios form 42.72 wt% and 57.28 wt% and 38.71 wt% and 61.29 wt%, respectively. The generated contribution margin for the aqueous phase for Scenario E and F was valued at 65.65 and 88.05 euro/ton. To reach the breakeven

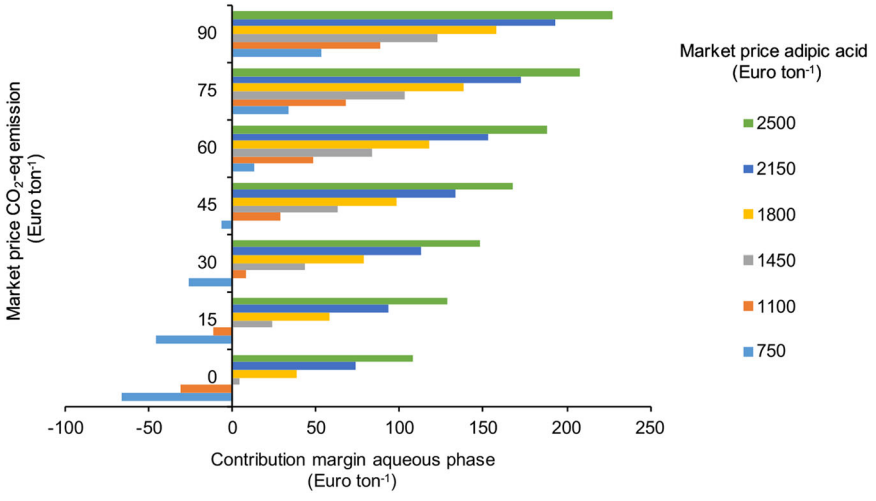

FIGURE 6 Technoeconomic sensitivity analysis for the aqueous phase of lignin bio-oil based on variable costs of Scenario F for the integrated biotechnological and chemical process to produce adipic acid accounting various market prices for adipic acid and for CO_2-eq emission. The calculation of the contribution margin of the aqueous phase of the bio-oil used the defined absolute yield and the concentration of the monomeric aromatics [Color figure can be viewed at wileyonlinelibrary.com]

point with a total direct production cost of 21.62–54.52 euro/ton (Tomani, 2010), as described for Lignoboost™, the organic oligomeric phase of the lignin bio-oil must generate a contribution margin of 45.16–247.82 and 24.86–274.83 euro/ton for Scenario E and F. Taking into account that exclusively the variable costs for adipic acid were calculated. From February 2016 to 2017, the market price for petrochemical phenol fluctuated between 1,130.50 and 1,607.00 euro/ton (ICIS Phenol Europe, UK). In addition, Biochar is obtained as an organic fertilizer, which corresponds to an absolute yield of 0.35 wt/wt of lignin (de Wild et al., 2014). Besides, pyrolysis gas can be used as an energy source. Also, it has been conceptually converted into biogas (91.1% CH_4) via anaerobic digestion with *Methanobacterium* (Li et al., 2017). Based on an overall expected contribution margin with the chosen approach, we conclude that an industrial-scale process is cost-effective. To achieve the proposed *cis*, *cis*-MA yield from true lignin-derived aromatics using whole-cell biocatalysts, further research is essential.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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