LIGNODECO Report Summary
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Final Report Summary - LIGNODECO (Optimized pre-treatment of fast growing woody and nonwoody Brazilian crops by detailed characterization of chemical changes produced in the lignin-carbohydrate matrix)

Executive Summary:
The primary objective of the LIGNODECO project is the development of optimized/new pretreatment technologies for deconstruction of high productivity and fast-growing Brazilian selected eucalypt clones and nonwoody lignocellulosic feedstock aimed at production of special pulps, biofuels and bio-materials, taking advantage from the use of modern analytical techniques, enabling in-depth identification of the changes produced in the main plant polymers and other minor constituents. The work plan encompasses seven work packages (WP1-WP7) broken down into twenty tasks, which resulted in twenty three deliverables. In WP1 the collection/selection of feedstock for deconstruction studies were dealt with. The optimization of pretreatments for woody and nonwoody materials was largely covered in WP2. Work package three (WP3) involves the in depth physical/chemical characterization of the original and pre-treated materials using advanced analytical tools. The tie in between pretreatment and industrial use of lignocellulosics is being studied under WP4. The demonstration studies are included in WP5 and the management and dissemination activities in WP6 and WP7, respectively. During the project were produced 3 patents, 13 peer-reviewed papers, 43 oral/visual presentations, and many other dissemination activities. The main results obtained so far include: (1) collection of about 16 tons of woody material derived from 19 double and triple crossing eucalypt clones and 2 tons of nonwoody material derived from 3 different elephant grasses; (2) the alkaline deconstruction pretreatments revealed that the NaOH+AQ process can potentially replace the kraft process both for woody and non-woody biomass; the eucalypt wood with greatest potential for NaOH+AQ deconstruction (high yield, low alkali demand) was the European Eucalyptus globulus, due to its highest lignin S/G ratio; the elephant grass was easy to deconstruct requiring low alkali and producing high yield an low rejects, even at high delignification degrees (kappa 50); (3) production of a low energy and high strength pulp with 6 % higher yield by deposition of xylans onto eucayptus kraft pulp in the oxygen delignification stage (patented); (4) production of a high strength eucalyptus kraft pulp through fiber treatment with a specially designed cellulase blend; development of a high biomass glucose-release LGF organosolv pretreatment assisted with phosphinic acid for bioethanol production (patented); (5) a high yield non-sulfur alkaline process adapted to lignin syringyl rich Eucalyptus globulus wood; (6) a medium sugar release bio-mechanical (pressafiner–laccase / mediator) pretreamatment for bioethanol production.

Project Context and Objectives:
Summary description of the project
Biomass production costs are low in Brazil compared to other parts of the world, due to proper climate and advanced forest and agricultural technologies. The productivities of elephant grass (Pennisetum spp; 30-45 t/ha/yr bone dry) and eucalypt crops (20-30 t/ha/yr bone dry) in Brazil are amongst the world largest, with good perspectives of growing even further. Thus, Brazil presents great potential for application of the Biorefinery concept. It is our contend that production of biofuels and bio-products from lignocellulosics is well fit to existing pulp manufacturing facilities, to take advantage of existing infrastructure with process integration. The objective of LIGNODECO is developing pretreatments for optimized deconstruction of hybrid
eucalypt clones and elephant grass biomass into its components aimed at production of biofuel (bioethanol and biogas) along with specialty grade pulps and other bio-products, strongly emphasizing feedstock selection and use of advanced analytical tools. This has been accomplished by collection/selection of high quality feedstocks for deconstruction studies, optimized pretreatments for woody and nonwoody materials, physical/chemical characterization of the pre-treated materials, and tie in between pretreatment and industrial use of lignocellulosics. LIGNODECO incorporates innovations such as tailor-made modifications of existing technologies for paper pulp industry, mild biotechnological pretreatments based on biocatalysts, modern analytical techniques for characterizing lignin, hemicelluloses and cellulose, utilization of Brazilian fast-growing woody and nonwoody crops as feedstock for future biofuels, novel high value-added cellulosic pulps from eucalypt and grasses for specialty papers, integrated use of xylan and lignin-containing by-products and effluents in energy and biogas production, and production of hemicellulose-based additives for wood pulps, and lignin-based chemicals and power/steam.

The LIGNODECO project aimed at developing new pretreatments for an optimised deconstruction of fast growing biomass into its main components to produce biofuels, along with pulp production for special paper grades and other bio-products. This approach required adequate analytical methodologies for the characterization of the structural and non-structural lignocellulose components, and their fate during the deconstruction process, especially when dealing with poorly characterized fast-growing feedstocks of interest for the Brazilian lignocellulose sector, such as the elephant grass and hybrid eucalypt clones. The following six innovative concepts were combined in the LIGNODECO proposal: i) Optimised deconstruction of lignocellulosic biomass by tailor-made modifications of already-available technologies used by the paper-pulp industry (such as alkaline cooking and a novel organosolv process); ii) New biotechnological pretreatments for separation of lignocellulose constituents under mild conditions, based on the use of enzymes as industrial biocatalysts, in combination with physical/chemical pre- or post-treatments; iii) Modern analytical techniques for characterization of lignin, hemicelluloses, cellulose and other biomass components, in raw materials, processes and products, as a knowledge-based way for developing optimised pretreatments; iv) Brazil fast-growing species of hardwood (eucalypt clones) and grasses (Pennisetum spp) as feedstock for future biofuels (bioethanol and biogas) production; v) Novel high value-added cellulosic pulps from eucalypt and grasses for specialty papers, including fibre functionalization and improvement; vi) Integrated use of xylan and lignin-containing by-products and effluents (in addition to cellulose use) in energy and biogas production, as well as to produce hemicellulose-based additives for wood pulps, and lignin-based chemicals.

The primary objective of the LIGNODECO proposal was developing optimised/new pretreatment technologies for deconstruction of high productivity and fast-growing Brazilian selected eucalypt clones and nonwoody lignocellulosic feedstock aimed at production of special pulps, biofuels and biomaterials, taking advance from the use of modern analytical techniques enabling in-depth identification of the changes produced in the main plant polymers and other minor constituents. With the purpose of attaining this primary objective, the following secondary objectives accomplished: (1) Collection/selection and general characterisation of the best woody and nonwoody lignocellulosic feedstocks that were used in the subsequent deconstruction studies; (2) optimisation of physical-chemical and enzymatic pretreatments adapted to rapid-growing feedstocks of interest for the Brazilian lignocellulose sector, including alkaline and organosolve pulping, together with use of hydrolases and oxidoreductases as deconstruction biocatalysts; (3) use of advanced analytical tools for in-depth characterization of pulp fibres, carbohydrates, lignins, black liquors and extractives during deconstruction of lignocellulosic biomass; (4) evaluation of the various pre-treated materials and fractions for production of special grade pulps with improved properties, additives, chemicals and biofuel with a new overall energy efficiency and waste and water minimization. The project was based on two lignocellulosic raw-materials - high-productivity and fast-growth Brazilian crops of clonal Eucalyptus and elephant grass (P. purpureum or Pennisetum americanum) - and combinations of enzymatic, chemical and/or mechanical pretreatments for cell-wall deconstruction for sustainable production of biofuels, specialty grade pulps and chemicals (including pulp additives) in such a way that the side streams and effluents were also processed for energy recovery (including biogas) and minimized water consumption. In-depth chemical characterisation of the different lignocellulose (structural and non-structural) constituents and their evolution at the three levels mentioned (raw materials, products and effluents) were crucial for developing knowledge-based deconstruction pretreatments.

The work plan encompassed seven work packages (WP1-WP7) broken down into twenty tasks, which resulted in twenty three deliverables. WP1 dealt with the selection of feedstock for deconstruction studies. The optimization of pretreatments was covered in WP2. WP3 involved the in depth physical/chemical characterization of the original and pre-treated feedstocks using
advanced analytical tools. The tie in between pretreatment and use of the lignocellulosic materials was realized in WP4. The demonstration studies were covered in WP5 and the management and dissemination activities in WP6 and WP7, respectively.

Work package 1 comprised the Collection, selection and general characterisation of the best woody and nonwoody lignocellulosic feedstocks to be used in the subsequent deconstruction studies. This work package was split into two tasks corresponding to the collection and storage (T1.1) and the general characterisation (T1.2) of woody and non-woody lignocellulosic feedstocks. In T1.1 samples of nineteen hybrid eucalypt clones and three high productivity elephant grasses were collected. After general characterisation of these woody and nonwoody feedstocks in T1.2 five hybrid eucalypt clones and one elephant grass species were selected. Thus, the subsequent work packages (WP2-WP4) were developed on six feedstock samples, five of eucalypt clones and one of elephant grass.

Work Package 2 aimed at optimizing physical-chemical and enzymatic pretreatments adapted to rapid-growing feedstocks of interest for the Brazilian lignocellulose sector. Four tasks were accomplished in this work package corresponding to chemical deconstruction of lignocellulosic feedstocks from WP1 by different alkaline processes, including kraft, soda-AQ, Soda-AQ-O2 (T2.1) by the LGF organosolve process catalysed with phosphinic acid (T2.2) and by biotechnological methods using hydrolases and oxidoreductases enzymes as deconstruction bioacatalysts, associated with mechanical treatment (T2.3). The treated materials and the isolated fractions obtained were submitted to in-depth characterisation in WP3, and evaluation (for biofuel, special-grade pulps, and other value-added products) in WP4. Work package 3 involved the use of advanced analytical tools for in-depth characterization of pulp fibres, carbohydrates, lignins, black liquors and extractives during deconstruction of lignocellulosic biomass. Four tasks were accomplished in this workpackage corresponding to analysis of fibre morphology and strength (T3.1); quantitative and qualitative analyses of polysaccharides (T3.2); quantitative and qualitative analysis of lignin and minor components (T3.3); quantitative and qualitative analysis of black liquors and other side streams (T3.4). Work package 4 involved the evaluation of the various pre-treated materials and cellulosic fractions for production of paper grade pulps with improved properties, additives, chemicals and biofuel with a new overall energy efficiency and waste and water minimization. Four tasks were accomplished in this work package corresponding to pulp characteristics and papermaking evaluation (T4.1) biofuel potential of the pre-treated material and residues (T4.2) upgrading xylans as paper pulp additives (T4.3) and optimised use of waste lignin (T4.4). Work package 5 involved pilot plant demonstration trials on organosolve pulping, biomass enzymatic pretreatment, ECF bleaching and pulp beatability. Three different tasks were accomplished. The most promising LGF organosolve treatment was successfully scaled up in the VTT pilot plant unit (Task T5.1). The enzymatic pretreatment and pulping/bleaching pilot plant for eucalypt and elephant grass (Task 5.2) were successfully accomplished in the Centre Technique du Papier (CTP) in Grenoble, France. The eucalypt and elephant grass pulp beatability trials (Task T5.3) were also successfully performed at CTP.

The following deliverables were accomplished in the course of the three-year LIGNODECO project: Collection of nonwoody and woody materials, general characterisation of elephant grass and eucalypt, selected woody (5) and nonwoody (1) feedstocks for pretreatment, wild-type and improved enzymes to be used in pretreatments, conditions for lignocellulose alkaline deconstruction, conditions for lignocellulose solvent deconstruction, pre-treated materials for characterisation and evaluation, enzyme action on models and lignin-carbohydrate complexes, isolated nonwoody xylan to be used as wood pulp additive, pretreatments conditions using selected enzymes, conditions for scaling up the enzymatic deconstruction, general characterisation of pre-treated materials using established analytical tools, set-up of advanced analytical methodologies for the pre-treated , characterisation of selected elephant grass and eucalypt using advanced analytical tools, characterisation of pre-treated woody and nonwoody materials using advanced analytical tools, characterisation of black liquors and other side streams, pulp and papermaking evaluation after optimised pretreatment, evaluation of pre-treated materials and residues for bioethanol and biogas production, setup of application method for anaerobic digestion and evaluation of pre-treated materials for biogas production, procedure for improving eucalypt pulp with grass xylan additive, evaluation of waste lignins for chemicals and energy, pilot-scale solvent deconstruction trials, pilot-scale enzymatic deconstruction trials and ECF pulp bleaching trials and pilot-scale pulp beatability trials.

Project Results:
The primary objective of the LIGNODECO project is the development of optimized/new pretreatment technologies for deconstruction of high productivity and fast-growing Brazilian selected eucalypt clones and nonwoody lignocellulosic feedstock...
aimed at production of special pulps, biofuels and bio-materials, taking advantage from the use of modern analytical techniques, enabling in-depth identification of the changes produced in the main plant polymers and other minor constituents. The following six innovative concepts were combined in the LIGNODECO goals:

i) Optimised deconstruction of lignocellulosic biomass by tailor-made modifications of already-available technologies used by the paper-pulp industry (such as alkaline cooking and a novel organosolv process);

ii) New biotechnological pretreatments for separation of lignocellulose constituents under mild conditions, based on the use of enzymes as industrial biocatalysts, in combination with physical/chemical pre- or post-treatments;

iii) Modern analytical techniques for characterizing of lignin, hemicelluloses, cellulose and other biomass components, in raw materials, processes and products, as a knowledge-based way for developing optimised pretreatments;

iv) Brazil fast-growing species of hardwood (eucalypt clones) and grasses (Pennisetum spp) as feedstock for future biofuels (bioethanol and biogas) production;

v) Novel high value-added cellulosic pulps from eucalypt and grasses for specialty papers, including fibre functionalization and improvement;

vi) Integrated use of xylan and lignin-containing by-products and effluents (in addition to cellulose use) in energy and biogas production, as well as to produce hemicellulose-based additives for wood pulps, and lignin-based chemicals. The work plan encompasses seven work packages (WP1-WP7) broken down into twenty tasks, which resulted in twenty three deliverables.

In WP1 the collection/selection of feedstock for deconstruction studies were dealt with. The optimization of pretreatments for woody and nonwoody materials was largely covered in WP2. Work package three (WP3) involves the in depth physical/chemical characterization of the original and pre-treated materials using advanced analytical tools. The tie in between pretreatment and industrial use of lignocellulosics is being studied under WP4. The demonstration studies are included in WP5 and the management and dissemination activities in WP6 and WP7, respectively. A summary with the main science & technology results obtained in the project are summarized below, but details can be found in the PDF Second Periodic Report attached in this final report.

1. WP1: Selection of the raw materials Work package 1 comprised the Collection, selection and general characterisation of the best woody and nonwoody lignocellulosic feedstocks to be used in the subsequent deconstruction studies. This work package was split into two tasks corresponding to the collection and storage (T1.1) and the general characterisation (T1.2) of woody and non-woody lignocellulosic feedstocks. In T1.1 samples of nineteen hybrid eucalypt clones and three high productivity elephant grasses were collected (Pennisetum purpureum of age 60 and 150 days and Pennisetum americanum of age 60 days). After general characterisation of these woody and nonwoody feedstocks in T1.2 five hybrid eucalypt clones and one elephant grass species were selected. Thus, the subsequent work packages (WP2-WP4) were developed on six feedstock samples, five of eucalypt clones and one of elephant grass.. With the exception of the European Eucalypts globulus, which was collected within the property of the ENCE company in Huelva, Spain, all other woody material was collected from an experimental station located in the city of Belo Oriente, Minas Gerais, Brazil. The experimental station is part of the Genolyptus project, a Brazilian nationwide research project to study eucalypt Genome. A total of nineteen wood samples including a twelve year old European Eucalyptus globulus (IG) and eighteen eucalypt hybrids, at the age of seven years, including a number of double/triple/fourth crossings among E. grandis, E. urophylla, E. globulus, Eucalyptus dunnii, E. camaldulensis species were collected (Table 1). Five representative trees of the population with average diameter at breast height (DBH) and height were selected. They were harvested and from each tree it was extracted five 1m-long bolts at 0, 25, 50, 75 and 100% of the three heights. A total of 25 bolts were collected for each wood type. The bolts were manually debarked, producing 13-15% bark on the overall wood weight. Table I: Description of the woody and nonwoody feedstock. #
Sample Code BiomassType 1 U1xU2 E. urophylla (Flores IP) x E. urophylla (Timor) 2 U2xC1 E. urophylla (Timor) x E. camaldulensis (VM1) 3 G1xUGL E. grandis (Coffs Harbour) x [E. urophylla (R) x E. globulus (R)] 4 U1xUGL E. urophylla (Flores IP) x [E. urophylla (R) x E. globulus (R)] 5 U1xC2 E. urophylla (Flores IP) x E. camaldulensis (VM2) 6 C1xC2 E. camaldulensis (VM1) x E. camaldulensis (VM1) 7 DGxUGL [E. dunnii (R) x E. grandis (R)] x [E. urophylla (R) x E. globulus (R)] 8 DGxU2 [E. dunnii (R) x E. grandis (R)] x E. urophylla (Timor) 9 C1xUGL E. camaldulensis (VM1) x [E. urophylla (R) x E. globulus (R)] 10 G1xGL2 E. grandis (Coffs Harbour) x E. globulus (R) 11 DGxCL1 [E. dunnii (R) x E. grandis (R)] x E. camaldulensis (VM1) 12 U2xGL1 E. urophylla (Timor) x E. globulus (R) 13 DGxGL2 [E. dunnii (R) x E. grandis (R)] x E. globulus (R) 14 U1xD2 E. urophylla (Flores IP) x E. dunnii (R) 15 U1xG2 E. urophylla (Flores IP) x E. grandis 16 IP E. urophylla (IP) x E. grandis (IP) commercial clone 17 VCE. urophylla x E. grandis commercial clone 18 CC E. urophylla x E. grandis commercial clone 19 IG E. globulus (Iberian) 20 EG 1 Pennisetum purpureum (150 days) 21 EG 2 Pennisetum americanum (60 days) 22 EG 3 Pennisetum americanum (150 days) Among the 18 eucalypt clonal and 3 elephant grass species, it was five woody and one nonwoody biomass that were used in the subsequent steps of the project. Nineteen eucalyptus wood clones and three elephant grasses were thoroughly studied. Out of these, the five eucalypt and one grass were selected on the basis of their field medium annual increment (MAI), density, fiber morphology and fiber chemical composition (carbohydrate, lignin content and S/G ratio, hemicelluloses, extractives, uronic acid and acetyl groups and minerals) for use in the subsequent steps of the project. Interesting differences were observed among the samples, for example, on the Examination of the cross sections of eucalyptus wood chips allowed visualising the vessels and fibres distribution in the annual growth ring and the vessels diameter were observed. The selected woody materials were: (1) a double crossing hybrid of Eucalyptus urophylla x Eucalyptus urophylla (U1 x U2), that was selected on the basis of its very high annual growth (83 m3/ha/yr), high wood density, excellent morphological traits, very high forest yield (43 ton/ha/yr) and low xylan and uronic acid contents; (2) a triple crossing hybrid of Eucalyptus grandis x (Eucalyptus urophylla x Eucalyptus globulus) (G1 x UGL), which presented a high xylan content and possessed Eucalyptus globulus in its genotype, which is of interest for Europe, although it is quite challenging for its high content of extractives (4.9%); (3) a triple crossing of (Eucalyptus dunnii x Eucalyptus grandis) x Eucalyptus urophylla (DG x U2), that was selected due to its highest annual growth (101 m3/ha/yr) among all eucalyptts evaluated, good density, outstanding morphological traits, and the highest forest yield (~50 ton/ha/yr); (4) a commercial elite clone (IP) obtained from a large Brazilian forest company, which is a double crossing of Eucalyptus urophylla x Eucalyptus grandis, for its excellent forest productivity (38.5 ton/ha/yr), good density, the highest cellulose content and lowest lignin content among all; also for being a very good reference since it is commercially planted by a large pulp company in Brazil. (5) A pure European Eucalyptus globulus, selected to attend EC request that at least one of the woody feedstock should originate from the European Continent; in spite of its lower MAI (~ 20 m3/ha/yr) in relation to the other feedstocks, the pure Eucalyptus globulus presented a very favorable chemical composition, including lower lignin and extractive contents and higher xylan content in relation to the other woody materials; it also presented a lignin S/G ratio over 4/1. The selected nonwoody feedstock was a 150 days old elephant grass of the Pennisetum purpureum species; it presented the highest productivity (32 ton/ha/yr) and density (216 kg/m3) at harvesting age (mature material), highest glucan content and lowest ash and uronic acid contents among the grasses evaluated. In general, the woody feedstock is less moist, denser and contains less minerals and extraneous materials than the nonwoody ones, which make them more attractive for utilization in deconstruction studies aimed at production of biofuels and bio-products. There is good correlation between soluble lignin content and lignin S/G ratio and this is important because soluble lignin quantification is much simpler than S/G ratio and could be a could indication for it. Although the criteria used to select the material is open to criticism and may not have been the wisest, it was the best the group came out with at our LIGNODECO sixth month meeting in Grenoble 2. WP2: Deconstruction pretreatments Work Package 2 aimed at optimizing physical-chemical and enzymatic pretreatments adapted to rapid-growing feedstocks of interest for the Brazilian lignocellulose sector. Four tasks were accomplished in this work package corresponding to chemical deconstruction of lignocellulosic feedstocks from WP1 by different alkaline processes, including kraft, soda-AQ, Soda-AQ-O2 (T2.1) by the LGF organosolve process catalysed with phosphinic acid (T2.2) and by biotechnological methods using hydrolases and oxidoreductases enzymes as deconstruction bioacatalysts, associated with mechanical treatment (T2.3). The treated materials and the isolated fractions obtained were submitted to in-depth characterisation in WP3, and evaluation (for biofuel, special-grade pulps, and other value-added products) in WP4. The main findings on this work package were: (1) the alkaline deconstruction pretreatment revealed that the NaOH+AQ process can potentially replace the kraft process for the European Eucalyptus globulus and the 150 days
woody and non-woody biomass, for a high degree of biomass delignification (kappa No. 15), without significant yield penalty and reject generation; (2) the different hybrid eucalypt wood clones behave similarly among themselves during the alkaline deconstruction treatments, but there was a significant advantage for the European Eucalyptus globulus in terms of alkali charge demand and process yield; (3) the elephant grass showed greater easiness of deconstruction than the woody materials and therefore its deconstruction shall be done at lower alkali charge and to higher delignification degrees than the woody ones; (5) the NaOH+AQ+O2 processes showed low potential for woody and nonwoody material deconstruction; (6) for low kappa numbers around which hardwood cooking is usually performed (approximately 15), the kraft process presented the highest screened yield values, irrespective of biomass used; (7) Soda-AQ and Soda-AQ-O2 presented screened yields comparable to the kraft process in the higher kappa range, indicating that these processes present good potential when woode deconstruction is aimed at biofuels and bio-products; (8) Kraft cooking performed the best among the cooking processes, but was closely followed by the Soda-AQ; (9) Eucalyptus globulus showed the best performance among the studied eucalyptus clones with respect to alkali charge and screened yield for kraft and Soda-AQ; (10) the LGF organosolv process applied to a hybrid eucalypt wood (triplet of E. grandis (G1) and E. globulus x urophylla (UGL)), and elephant grass EG1 (Pennisetum purpureum) produced hydrolysable biomass at high yield; (11) ethanol is well applicable solvent for both hardwoods and grasses; (12) depending on the used conditions, LGF organosolv cooking produced fiber fractions with high yields of 64-96%; (13) In the cases with highest yields (without any catalyst, at lower cooking temperature or at lower water content), both delignification and defibration after LGF cooking was limited; (14) In the cases with highest yields (without any catalyst, at lower cooking temperature or at lower water content), both delignification and defibration after LGF cooking was limited; (15) in LGF processes pulp xylan proportion decreases with increasing phosphinic acid charge; (16) when the pulp polysaccharide contents are calculated to the total pulp yields, it can be estimated that 40-50 % of original wood raw material can be recovered as cellulose available for bioethanol production; (17) based on chemical compositions, sufficient deconstruction of eucalypt could be reached with phosphinic acid charge of 3.5-5% at 130 °C and 15% water content during fixed 48h cooking time. Best hydrolysis results were also obtained in these conditions, as reported in WP4. For industrial processes shorter cooking times must however be reached; (19) due to the lower density of elephant grass, LGF with shorter cooking times are possible; (20) After LGF cooking pulp yields of 55-75% were obtained, and pulp lignin contents varied from 15.3-10.9%; (20) with increasing LGF cooking time, both the xylan and cellulose yields decreased; (21) lowest lignin content was reached with U1xU2 clone, whereas the highest cellulose yield was retained with the IP hybrid; (22) alkaline extraction after LGF cooking further improved the glucose yield for both eucalyptus and elephant grass; (9) best results for eucalyptus G1xUGL were obtained at cooking temperature of 130°C, water content of 15% and phosphinic acid charge of 3.5% and 48 h cooking time (for EG a 24-32h cooking time was sufficient); (23) some hemicelluloses degradation occurred during LGF cooking, especially with high phosphinic acid charges or long reaction times; (24) in optimised LGF cooking conditions (3.5% H3PO2, 15% water, 130°C), sufficient delignification with higher polysaccharide yield could be obtained already during 20h cooking. To reach industrially applicable process, further reduction in LGF cooking time could be possible by impregnation, more efficient mixing/liquor circulation, higher temperature or using fresh chips; (25) the LGF cooking after 20h and alkaline extraction, the DGxU2 clone with highest pulp yield seemed most potential raw material for bioethanol production; (26) the xylanase-based product, NS-51115, for the pretreatment of eucalyptus wood prior to mechanical deconstruction; (26) the lignin content in both lignocellulosic samples decreased after the enzymatic treatments concomitantly with increasing laccase doses; (27) in Elephant grass, the lignin content decreased about 11, 22 and 32% after the laccase-mediator treatments using enzyme doses of 10, 25 and 50 U/g, respectively; (28) the reduction in lignin content of eucalypt wood samples was more pronounced than grass, attaining 32, 34 and 48% lignin decrease using laccase doses of 10, 25 and 50 U/g, respectively; (30) the treatments with laccase alone (without mediator) scarcely decreased the lignin content (<5%) in both materials (eucalypt and grass); (31) there is a general tendency at increasing enzyme (laccase) doses is a significant decrease of lignin carbon (in aromatic, side-chain and methoxyl structures), although in a lower extant than in the Elephant grass samples, and a concomitant increase of polysaccharides (including both normal and acetylated units); (32) the effect of the increase of mediator doses was studied after 6 hours reaction. However, when increasing the doses of MeS as high as 12%, (and using 50 U/g MtL) only minor lignin removal (16% lignin decrease) was observed; (33) Also, the presence of organic solvents, such as methanol barely modified the lignin removal, even when large reaction times (24 hours) were used for the enzymatic treatments; (34) the lignin content of eucalypt wood feedstock after each cycle of enzymatic treatment using MtL and MeS as
mediator indicated that the delignification increases with the number of sequences used; (35) the results obtained indicate, for the first time, that the laccase–mediator treatment using a low-redox laccase and a natural phenolic compound as mediator, that produces up to 50% lignin reduction of eucalypt wood feedstocks, can be an economically feasible procedure from an industrial point of view, since both the laccase (MtL) and the phenolic mediator (MeS) used are commercial and cheap; (36) in addition to assessing the lignin content decrease, further insight into the lignin structure modification by the enzymatic treatments was achieved by 2D NMR analyses of the lignocellullosic samples; (37) the HSQC spectra of the eucalypt wood after the enzymatic treatments with different laccase doses showed important differences compared to the control ones, being that the signals of side-chains in β-O-4’ lignin substructures (A) present in the aliphatic oxygenated region of the spectrum strongly decreased after laccase-mediator treatment. Likewise, signals of S lignin units present in the aromatic region of the spectrum also strongly decreased after the laccase-mediator treatment with respect to the control, and the cross-signal of oxidized S-lignin units (S’2,6) increased concomitantly; (38) the results obtained indicate that the laccase- mediator treatment using a low-redox laccase and a natural phenolic compound as mediator, that produces up to 50% lignin reduction of eucalypt wood feedstocks, can be an economically feasible procedure from an industrial point of view, since both the laccase (MtL) and the phenolic mediator (MeS) used are commercial and cheap; (39) slightly more buffer-soluble material is produced from EG by the MSD Pressafiner (12%) than is commonly present (10%); (40) the Pressafiner pretreatment had no apparent effect on fibre solubilisation by Ultraflo, while with Cellulase NS-22086, 3.5% more EG-P biomass was solubilised compared to the EG sample; (41) when Celluclast was used instead of NS-22086 for the hydrolysis, both in the absence and presence of BG, the extent of solubilisation of EG was up to 5% higher than that of EG-P, the converse of that seen with the NS-22086 preparation; (42) NS-22086 has a similar origin to Celluclast but the hydrolytic activity is further boosted by the addition of more cellulolytic and hemicellulolytic activities, making it more suitable for biomass breakdown; (43) Ultraflo was demonstrated to be the poorest performing enzyme preparation on EG of the three preparations examined on an xylanase-equivalent dosage, as with biomass solubilisation, there was no increase in reducing group released upon hydrolysis after Pressafiner pretreatment; (44) the amount of reducing groups generated through hydrolysis of the polysaccharides in the biomass was lower with EG-P compared to the untreated EG. It is possible that hydrosolubles present in the Elephant grass are generally easily solubilised and higher reducing group production is a result of further degradation of this soluble material by the enzymes within the Celluclast preparation. Alternatively, pretreatment with the Pressafiner is generating inhibitory agent(s) of enzyme action. The higher level of reducing ends generated by Celluclast treatment in the presence of BG is due to the further hydrolysis of the reaction products by the endo-acting enzymes; (45) the Pressafiner treatment enabled some modifications in the fibres that affect enzyme accessibility and, in the case of NS-22086 and Ultraflo, the higher esterase activities present in the preparation with respect to Celluclast are helping in the material solubilisation and acetate release; (46) it is clear that NS-22086 is the most effective among the enzyme preparations in the removal of glucan from the lignocellulosic matrix, and that surprisingly, the addition of the β-glucosidase preparation (NZ-188) resulted in a poorer solubilisation of the available glucose in EG, as well as the poorest degree of solubilisation (49.5%); (47) the residual Klason lignin was not significantly different between the four samples, demonstrating that these enzymes do not act upon this polymer, but the amount of recovered acid-soluble lignin (ASL) was reduced by the action of NS-22086 and Celluclast; (48) the main sugar removed through the action of these cellulolytic cocktails was not surprisingly glucose, but what was surprising was that arabinose- and xylose-containing polysaccharides within EG were most resistant to the action of these enzymes; this demonstrates that the hemicellulosic material present within the EG matrix does not form a barrier to the removal of glucan; (49) the time-course of hydrolysis of Elephant grass by Celluclast and Pressafiner-treated Elephant grass by Cellulase NS-22086 showed a rapid increase in biomass solubilisation and reducing sugar release during the first 3-10 h; after 10 h, little change is observed in all measured parameters for both substrates (EG and EG-P), over the 10-d time course; (50) still on the time-course of hydrolysis of Elephant grass by Celluclast and Pressafiner-treated Elephant grass by Cellulase NS-22086, the increase in reducing sugar levels was higher for the EG-P sample digestion with NS-22086 during the first 12 h, but after 2 3 days similar values were observed for both sample-enzyme treatments, released acetic acid levels reached a more or less constant value after 12-h incubation of both samples, the levels being higher for the EG-P sample treated with NS-22086; (51) Overall, extracted EG pith is easier to degrade than the whole stem or cortex/bark as shown by the increased biomass solubilization observed specially with Ultraflo and NS22086 compared to the whole fibre although the effect was not so evident when using Celluclast (yielding similar solubilization values for whole material and pith); (52) It has been shown on wheat
straw that degradation occurs from inside to outside and the vascular bundles located at the outer edge of the cortex together with the cuticular epidermis are not significantly affected by the enzymes; the presence of the pith in EG reduces this accessibility to the cortex further resulting in poor degradation, and with the presence of less accessible areas on the pith in the whole stalk material will also reduce degradation compared to the exposed pith samples; (53) organic co-solvents can expand the use of enzymes in lignocellulose deconstruction through making hemicelluloses and lignin more soluble or at least less compact due to disruption of hydrophobic and electrostatic bonds between the polymers and thus more accessible to enzymatic degradation; (54) appears that the use of DMSO does not improve biomass solubility, and generally leads to a reduction in degradation. However, DMSO addition did not significantly affect the reducing sugar or acetic acid release by Ultraflo, the latter values being slightly enhanced in whole EG and cortex fraction indicating that xylanase and esterase activities in Ultraflo could be stimulated by the presence of DMSO, which compliments the results reported previously on the stimulation of feruloyl esterase activity on model compounds by the presence of 20% DMSO, as well as the protective effect of DMSO on xylanase stability throughout the reaction time course; (55) the removal of glucose with DMSO does to correspond to the lower degree of solubility in the presence of DMSO, suggesting that the co-solvent is restricting more the removal of extractives during the hydrolysis treatment; (56) a range of experimental and commercial enzymes, notably multi- and monocomponent carbohydrases and ferulic acid esterases, assembled with consideration of eucalyptus and elephant grass compositional data have been developed by NOVOZYMES and supplied to relevant consortium members; (57) on the enzymatically assisted deconstruction pretreatment it was concluded that ultraflo solubilizes a high proportion of lignocellulosic biomass with concurrent release of reducing groups, and that the new cellulase NS-22086 was found to solubilize more whole elephant grass (in 72 h) than Ultraflo; (58) the supplementation of Ultraflo with pure feruloyl esterases increased the amount of solubilisation only by a few percentiles;(59) the pressafiner device proved effective to impregnate enzymes into the elephant grass sample; (60) it was done the chemical surface mapping of eucalyptus and elephant grass pulps for xylan was carried out by immunochemical labelling, were obtained interesting results showing the deposited xylans; (61) the laccase-mediator system was applied on the eucalypt wood and elephant grass materials to degrade and/or modify the structure of the lignin; (62) two laccases, with high and low redox-potentials (obtained from Pycnoporus cinnabarinus, PcL, and from Myceliophthora thermophila, MtL, respectively), were assayed in combination with synthetic (1-hydroxybenzotriazole -HBT, violuric acid -VA) and natural mediators (Methyl syringate -MeS), respectively; (63) Optimization of the laccase-mediator treatment, including a subsequent alkaline peroxide stage in four cycles treatment in an oxygen pressurized reactor, resulted in important lignin removal from both elephant grass and eucalypt wood (up to 30-45% lignin reduction); (64) Comparing the data from elephant grass and eucalypt wood, it seems that woody materials have a better behaviour for the enzymatic removal of lignin. Analyses by 2D-NMR confirmed the strong modification of the lignin structure during the treatment of eucalypt wood and elephant grass with the laccase-mediator system. Thus, these results open up new mechanisms for the enzymatic deconstruction of different woody and non-woody raw materials; (65) Elephant grass pith is more readily solubilised than the bark portion, even though chemically there are very similar; (66) mechanical pretreatment of whole elephant grass with a Pressafiner led to an increase in biomass solubilisation with a Cellulase preparation but did not improve Ultraflo hydrolysis. The improvement is thought to be due to an opening of the matrix fibre structure allowing better penetration of enzymes to their sites of hydrolysis; (67) the lack of improvement with Ultraflo suggests that the enzymes in that mixture can access the hydrolysis sites without further pretreatments but the types of enzymes present are not as suitable to elephant grass hydrolysis as with the Cellulase preparation. A significant release of acetic acid release was observed with both Cellulase and Ultraflo treatment after Pressafiner, further demonstrating that acetate substitutions are not a barrier to enzyme hydrolysis of lignocellulosics; (68) the study of the delignification of elephant grass and eucalypt wood by the use of a high-redox potential laccase (from Trametes villosa; TvL) in the presence of HBT as mediator showed for the first time that woody and nonwoody biomass can be significantly delignified by this laccase-mediator system (with 30-50% lignin removal) by applying a sequence consisting of several alternative laccase-mediator and alkaline extraction stages, directly on the whole lignocellulosic material. The enzymatic pretreatments also resulted in improved cellulase hydrolysis, enabling shorter fermentation times for ethanol production; (69) the lignin content in elephant grass was not modified by the use of this low-redox laccase-mediator system. However, and interestingly, when applied to eucalypt wood, the lignin content dramatically decreased after the enzymatic treatments, attaining up to 50% lignin removal when using high doses of MtL and MeS; (70) enzymatic pretreatment with hydrolases, the Novozymes cellulolytic enzyme cocktail NS-22086 was found to be the most efficient preparation for the
solubilisation of finely milled elephant grass (EG), especially after the biomass has been modified slightly by MSD Pressafiner treatment. An optimum dosage (100 ml/kg biomass), pH of 6 and the reaction to take place in water was recommended for the demonstration trials at CTP (WP5); (71) for biogas production a considerable recalcitrance of raw eucalyptus to anaerobic digestion (~8% of the carbohydrate fraction converted to biogas) thereby indicating that some form of pretreatment (or blending/co-digestion) will be essential to extract energy value in the form of biogas from this particular raw feedstock; (72) the establishment and validation of the previous described technique enabled us to identify a novel xylanase-based product, NS51115, among more than 30 enzymes and enzyme blends, both commercial and experimental, for the pretreatment of eucalyptus wood prior to mechanical deconstruction. This candidate repeatedly showed to have a destabilizing effect on the cell wall structure and was chosen for the optimization trials which generated a prediction model based on 120 data points revealing the optimum conditions; (73) several enzymatic fiber modification trials have been conducted with the unconventionally deconstructed materials. Most of the deconstructed materials were readily modified by the cellulase treatments and the prepared handsheets showed improvement in tensile strength. Specifically the Cel45A and Cel7b endoglucanase families demonstrated the ability to improve resultant handsheet physical strength properties, the latter enzyme treatment without sacrificing tear strength; (74) Also the Soda-AQ E. globulus fiber (both kappa 15 and 20) showed to be susceptible to enzymatic strengthening, resulting in tensile strengths equal to the otherwise stronger Kraft fiber, thus enabling a completely sulphur-free pulping process. Refining trials carried out on the selected Soda-AQ E. Globulus pulps did not reveal any significant difference in the physical properties between the kappa 15 and 20 pulps, except in tear strength where the kappa 20 pulp was superior. The enzymatic treatments of these pulps with both Cel45A and Cel7b revealed the need for further process optimization in order to obtain the expected benefits, although large increases in freeness developments were identified as well as increases around 10% in tensile strength after 1000 PFI rev; (75) Furthermore these unconventional pulps were investigated with regards to their susceptibility enzymatic delignification (i.e. bleaching) by the application of oxidoreductases both alone and in combination with mediators. These trials revealed that the high redox-potential laccase from Polyporus pinsitus showed synergistic behaviour when combined with violuric acid, reaching a brightness increase of 16 units for the kappa 20 pulp. Also interesting was it that the same absolute brightness values were obtained when kappa 15 and kappa 20 pulps were subjected to the enzymatic bleaching, which can be translated into increased pulp yield and cost savings on chemicals; (33) The enzymatic delignification system was further optimised with the Coprinus cinereus peroxidase combined with violuric acid and it was shown that this system increased brightness by 7 units at a very low dosage of 0,3 mM of violuric acid and 0,5 mM of hydrogen peroxide; (76) it was also shown that conventional bleached Kraft pulp with little effort could be converted into dissolving pulp of a proper grad via enzymatic routes. The application of xylanases and cellulase had positive influence on the solubilities (S10 and S18) and the intrinsic viscosities of the investigated pulps, thus enabling a regular kraft mill to obtain dissolving pulp grades without the need for a pre-hydrolysis step. 3. WP3: Physical/chemical characterization of the original and pre-treated materials using advanced analytical tools Work package 3 involved the use of advanced analytical tools for in-depth characterization of pulp fibres, carbohydrates, lignins, black liquors and extractives during deconstruction of lignocellulosic biomass. Four tasks were accomplished in this workpackage corresponding to analysis of fibre morphology and strength (T3.1); quantitative and qualitative analyses of polysaccharides (T3.2); quantitative and qualitative analysis of lignin and minor components (T3.3); quantitative and qualitative analysis of black liquors and other side streams (T3.4). The main findings during the characterisation of selected raw materials using advanced analytical tools were: (1) the morpholgical characterization of the pretreated materials before and after an ODIPD bleaching sequence, and the main findings among the five selected hybrid eucalyptus clones were: (a) IP and E. globulus eucalyptus woods were the most interesting raw materials for Kraft pulp manufacture, IP pulp presented the longest and flexible fibres with a high bonding potential, and E. globulus pulp has the lowest vessels content and the longest fibres; (b) DGxU2 eucalypt hybrid seemed to be one of the most interesting raw material for pulp manufactured with Soda-AQ process; (c) after Kraft cooking, tendencies observed on pulps were not similar as tendencies observed on the raw materials; (c) the best process depended on the raw materials. Soda-AQ cooking seemed to be the most interesting process for the manufacture of E. globulus and DGxU2 pulps; this cooking induced a reduction of vessels content and an improvement of the fibre flexibility and seemed to reduce the broken fibres content compared to the Kraft process; (d) Kraft cooking was the most interesting process for the manufacture of IP eucalyptus pulp; the IP Kraft pulp contained longest fibre with bonding potential and lower vessels content than the IP pulp manufactured with Soda-AQ process; (e) morphological analysis on elephant grass
pulp (EG1) showed that Soda-AQ process was slightly more interesting than Kraft cooking, since this process allowed reducing vessels content in the pulp and fibres were more flexible. However, hydrogen bonding potential was higher after Kraft process; (2) the lignin from the eucalypt hybrids IP, U1•U2 and DG•U2 present a very similar lignin composition (28-30%) and S/G ratio (2.4-3.0) among themselves; while the lignin from G1•UGL and the European Eucalyptus globulus present the highest S/G ratios. 3.6 and 4.0 respectively. (3) For all eucalypt woods the main lignin substructure present was the β-O-4’ alkyl aryl ether (75-79% of all side-chains) followed by the resinol substructure (9-11% of all side-chains) and the other linkages, such as phenylcoumaran, spirodienone or β-1’ open substructures were present in lower proportions (1-5% of all side-chains); (4) the lignin from the hybrid G1•UGL and European Eucalyptus globulus also presented the highest proportion of β-O-4’ linkages; (5) the residual lignins isolated from G1xUGL pulps prepared by the soda-AQ and soda-O2 processes were β-O-4 aryl ether, with lower amounts of β-β resinol and β-5 phenylcoumaran structures, as also observed in the native lignin; (6) the main lignin substructures present in the residual lignins isolated from elephant grass pulps prepared by the soda-AQ and soda-O2 processes were β-O-4 aryl ether, with lower amounts of β-β resinol and β-5 phenylcoumaran structures, as also observed in the native lignin; (7) in the case of the eucalypt wood, the residual pulp lignin are enriched in H- and G-lignin units, and depleted in S-units with decreasing kappa number. Moreover, there is also an increase in the amounts of lignin pyrolysis compounds with shorter chain, indicating a partial lignin degradation with decreasing kappa number; (8) in the case of the elephant grass, the residual pulp lignin are also enriched in H- and G-lignin units, and depleted in S-units with decreasing kappa number; moreover, there is also an increase in the amount of lignin pyrolysis compounds with shorter chain, indicating a partial lignin degradation with decreasing kappa number; (9) comparing soda-AQ and soda-O2 processes, it is apparent that, at the same kappa number, the soda-O2 process produces pulps with lower content of β-O-4 aryl ether linkages for the pulps with higher kappa; therefore, it seems that the soda-O2 process is more efficient than soda-AQ process for delignification of eucalypt wood, at least at high kappa numbers; (10) the most predominant lipophilic extractives compounds present in all the eucalypt woods were steroids, including sterols, sterol glycosides and sterols esters with lower amounts of steroid ketones and steroid hydrocarbons; (11) the wood from U1•U2 had the lowest content of these detrimental compounds while the woods IP and DG•U2 had the highest content of them and therefore it is foreseen that the latter woods will have more pitch problems than the former one; (12) the analyses of the elephant grass indicate that the composition of the lignin is quite similar in its bark and pith components, with similar S/G ratios (1.45 and 1.49 respectively) and a predominance of β-O-4’ units (77-79% of total side-chains). With lower amounts of condensed linkages such as β-5’ phenylcoumaran-type (7-8%). β-β’ resinol-type units (4-2%) and β-1’ spirodienone-type units (3%) and the lipids from the bark and pith of the elephant grass accounted for 0.98 % and 1.56% of the total sample, although the general composition was similar; (13) soda-AQ pulps from both woody and nonwoody feedstocks showed a higher content of glucans an lower contents of xylans in relation to kraft; (9) The European Eucalyptus globulus (IB) showed higher xylans content in all deconstruction treatments in relation to the other woody materials; (14) the surface distribution of xylan was not even in the fibers of alkaline pulp, and higher xylan contents could be detected by labelling especially in damaged fibres, fibrillated fines and around pits. After reprecipitation of EG xylan, the overall labelling of bulk fibres was increased, and especially in fine fibrils and fibre defects. Some fibres were very evenly and heavily labelled; (15) Detailed structural characterization of lignin and lignin-carbohydrate complexes extracted from European Eucalyptus globulus and 150 days old Pennisetum purpureum using a combination of spectroscopic (2D-NMR) and degradative (analytical pyrolysis, thioacidolysis and derivatization followed by reductive cleavage) methods revealed important changes in lignin composition and structure with three aging including increase of S/G ratio and ether-bond frequency were observed; in the case of the elephant grass; the preliminary analysis of lignin revealed the presence of p-coumaric acid and acetic acid moieties acylating the phenylpropanoid units at the δ-position; the fractionation of lignin-carbohydrate complexes (LCC) from eucalypt and elephant grass revealed two fractions. LCC1 enriched in cellulose with a low lignin content and LCC2 enriched in xylan and with high lignin content; the procedure of LCC fractionation hydrolysates the acetyl groups in the hemicelluloses and in the lignin; (16) analyses by 2D-NMR confirmed the strong modification of the lignin structure during the enzymatic treatment; enzymatic deacetylation of whole fibers was more apparent where the acetate was associated with the hemicellulosic fraction rather than the lignin; (17) MWL could be separated into a “pure” lignin and a “lignin-carbohydrate” (LCC) portion; 2D-NMR demonstrated that the enzymes did not deacylate the “pure” lignin material, but the carbohydrate portion of MWL was reduced by 80% with the ultraflo; (18) the main linkages observed in the residual lignins from G1xUGL and elephant grass, in both the kraft and soda-AQ pulps, were β-O-4 aryl ether, with lower amounts of β-β
resinol and β-5 phenylcoumaran structures; a reduction of the main substructures was observed after the cooking, this reduction being more evident in the pulps with lower kappa number (kappa 15) due to the higher extent of delignification; (19) at similar kappa numbers, the content of β-O-4 aryl ether linkages in the residual lignins from G1xUGL and elephant grass were lower for the soda-AQ process than for the kraft process, indicating a higher efficiency of the soda-AQ process for delignifying these feedstocks, in the case of the pulps intended for bioethanol and biogas production, it seems that the soda-O2 process is more efficient than soda-AQ process for delignification of eucalypt wood and elephant grass; (20) cellulose crystallinity was not shown to correlate with hydrolysability of LGF and alkaline pulps, and other factors, e.g. lignin and xylan content seem to affect more; (21) structural changes in lignin revealed that also in LGF cooking the syringyl type units are more reactive, and condensation increases during cooking. PA is essential in these reactions; (22) In alkaline cooks, the residual lignin of Soda-O2 pulp was less phenolic, which may restrict the reactivity in following bleaching stages if aimed at paper pulp. Also in this respect the method is more suitable for the bioethanol production; (23) Most phenolic syringyl units were formed in Soda-AQ cooking, suggesting higher reactivity of the pulp lignin. In all the alkaline cooks, the condensation reactions were most extensive at the end of the cook; (24) Two different sets of pulps produced from elephant (soda-AQ and soda-O2 processes) grass and eucalypt hybrid G1xUGL by different cooking processes were received from Suzano: i) Pulps intended for paper production (pulp samples from eucalypt G1xUGL and elephant grass prepared by the kraft and soda-AQ processes at kappa 20 and 15, and their respective black liquors); and ii) Pulps intended for bioethanol and biogas production (pulp samples from eucalypt G1xUGL and elephant grass prepared by the soda-AQ and soda-O2 processes at kappa 50, 35 and 15); (25) the main linkages observed in the residual lignins from G1xUGL and elephant grass, in both the kraft and soda-AQ pulps, were β-O-4 aryl ether, with lower amounts of β-β resinol and β-5 phenylcoumaran structures; (26) a reduction of the main substructures was observed after the cooking, this reduction being more evident in the pulps with lower kappa number (kappa 15) due to the higher extent of delignification. At similar kappa numbers, the content of β-O-4 aryl ether linkages in the residual lignins from eucalypt G1xUGL and elephant grass were lower for the soda-AQ process than for the kraft process, indicating a higher efficiency of the soda-AQ process for delignifying these plant feedstocks; (27) in the case of the pulps intended for bioethanol and biogas production, it seems that the soda-O2 process is more efficient than soda-AQ process for delignification of eucalypt wood and elephant grass; (28) The establishment and validation of the previous described technique enabled us to identify a novel xylanase-based product, NS51115, among more than 30 enzymes and enzyme blends, both commercial and experimental, for the pretreatment of eucalyptus wood prior to mechanical deconstruction. This candidate repeatedly showed to have a destabilizing effect on the cell wall structure and was chosen for the optimization trials which generated a prediction model based on 120 data points revealing the optimum conditions; (29) regarding the chemical composition of the lipophilic extractives, the most predominant compounds present in all the eucalypt woods were steroids, including sterols, sterol glycosides and sterols esters with lower amounts of steroid ketones and steroid hydrocarbons; other important lipophilic compounds found were series of fatty acids, glycerides (including mono-, di- and triglycerides) and minor amounts of squalene, tocopherols (in free and esterified form) and a series of alkylferulates; (30) the high amounts of neutral compounds in most of these woods, and particularly the high abundances of free and conjugated sterols, which have a high propensity to form pitch deposits would point to a high pitch deposition tendency of the lipophilics from these woods; (31) among the eucalypts, the wood from U1[JU2 has the lowest content of these detrimental compounds and therefore will have less pitch problems, while the woods IP and DGU2 have the highest content of them, and therefore it is foreseen that they will have more pitch problems than the woods from the hybrid U1[JU2; (32) regarding the grass, the lipids from the bark and pith accounted for 0.98 % and 1.56% of the total sample, although the general composition was similar, being the most predominant lipids in the bark and pith are fatty acids and free sterols. Additionally, significant amounts of steroid ketones, triglycerides, a series of feruloyl esters, waxes as well as steroid hydrocarbons and sterol esters, were also present. Sitosterol was the most important compound identified in the elephant grass extracts, together with other free sterols such as campesterol and stigmasterol. These sterols were also found esterified with long chain fatty acids; (33) a protocol based on the solubilization of the whole lignocellulosic material in DMSO:TBAH and subsequent precipitation in water, was developed. Two different LCC fractions were separated, one enriched in cellulose and depleted in lignin (a glucan-lignin complex) and the other enriched in lignin and hemicelluloses (a xylan-lignin complex). However, the analyses also indicated that the acetyl groups from the hemicelluloses, as well as those acetyl groups attached to the γ-carbon the lignin, are hydrolyzed and removed during the fractionation protocol; (34) regarding black liquor investigation it was observed that different pulping
processes and raw materials result in black liquors with distinct chemical compositions that must be considered upon their utilization, being that the main findings on black liquor characterization were: (a) the lignins precipitated from the black liquors were analysed by NMR and Py-GC/MS and it was observed that the lignin in black liquors is completely different from the native lignin and from the residual lignin in the pulp; the lignin in black liquors is highly enriched in S-lignin units (due to the preferential solubilisation of S-lignin units) and depleted in G-units; in addition, the higher amounts of pyrolysis compounds with shorter chain and/or oxidized, indicates that this lignin is heavily degraded and oxidized; in the case of elephant grass, important amounts of H-units, arising from p-coumaric acid, can also be observed, indicating that p-coumaric acid is present in free from or etherified to the lignin; (b) it is clear from the NMR spectra that these precipitated lignins from black liquor are enriched in β-β resinol structures, while the other linkages (β-O-4 alkyl-aryl ether and β-5 phenylcoumarans), if present, are in much lower amounts. An increase of the S/G ratio is observed, that indicates that S-lignin units, which are predominantly forming β-O-4 alkyl-aryl ether structures, are preferentially removed from the eucalypt during pulping and are being enriched in the black liquors; (c) It is interesting to note that the lignins from soda-AQ process are more enriched in S-lignin units that the lignins from kraft process. In addition, while minor amounts of β-O-4 alkyl-aryl ether structures are still present in the lignins from kraft process, they were completely absent in the lignins from soda-AQ process; (d) Therefore and as already observed in the analysis of the residual lignins from these pulps, the comparison between the kraft and soda-AQ process indicates that the latter process seems to be more efficient to depolymerize the lignin from eucalypt wood than the kraft process; (e) lignins precipitated from the black liquors from the pulping of elephant grass by the kraft and soda-AQ processes at kappa 20 and kappa 15 were also studied by 2D-NMR and the result showed the p-coumarate esters in the γ-carbon have been completely hydrolyzed, and the p-coumaric acid has been liberated. However, only in the black liquor from kraft kappa 20, important amounts of p-coumaric acid could be detected. β-O-4 linkages are only present in low amounts, and its abundance are reduced with decreasing kappa number due to the more drastic pulping conditions. Comparing kraft and soda-AQ processes, the abundance of β-O-4 linkages is lower, in the latest, which seems to indicate that soda-AQ process performs more efficiently in elephant grass, as also occurred with eucalypt woods; (f) condensed structures, such as β-β resinols and β-5 phenylcoumarans, are present in relatively more abundance in the black liquor compared to the MWL, and especially the resinols, as also occurs in eucalypt wood, despite its very low abundance in the native lignin; (g) the S/G ratio is higher in the lignins precipitated from the black liquors, which indicates that S-lignin units, that form predominantly β-O-4 linkages are preferentially removed during cooking. The higher S/G value observed in the liquors of the soda-AQ process compared to the kraft process is another indication of the better performance of soda-AQ for delignifying elephant grass, as already observed in eucalypt wood; (h) the black liquor sulfur content is lower for the pulp produced at kappa 20 than at kappa 15; (i) the black liquor gross heating value is superior for pulps produced at kappa 20 than at kappa 15; (j) the gross heating value of black liquor derived from Soda-AQ process is higher than that derived from kraft process; (k) the black liquor derived from soda-AQ pulping of elephant grass has lower heating value than that from wood at a given kappa number; (m) Black liquor derived from pulping of elephant grass has lower carbon content and unusually high nitrogen content when compared to wood black liquor; (n) when cooking eucalyptus wood to kappa 20, a small but significant amount (8-10%) of xylans of reasonable MW remains in the black liquor; (o) recovery of such xylans (~1.5% on pulp weight) is possible by adding black liquor to the oxygen delignification stage (O-stage) resulting in yield gain of the same proportion; (p) Such practice impairs the O-stage performance only slightly but impairs bleaching significantly (+2 kg ClO2/odt); (q) yield and pulp quality gains must be weighed against increased ClO2 demand for pulp bleaching; (r) All the materials could be considered for energy purposes, even though some of them present better high heating value, giving a higher energy production; (s) The characterization of the organic and inorganic content of each side stream didn’t present any material that should be a concern or could hinder the use and application; (t) Only when we think in a high scale process in continuous looping and closed system the inorganic content could hinder the production and jeopardize the investment; (u) lignin could be used in several applications, and its sustainable source is only one of the good aspects of it as a product. Also its properties provide several applications and can have a good value as a commercial product. 4. WP4: Tie between pretreatment and industrial use of lignocellulosics Work package 4 involved the evaluation of the various pre-treated materials and cellulosic fractions for production of paper grade pulps with improved properties, additives, chemicals and biofuel with a new overall energy efficiency and waste and water minimization. Four tasks were accomplished in this work package corresponding to pulp characteristics and papermaking evaluation (T4.1) biofuel potential of the pre-treated material and residues (T4.2) upgrading xylans as paper pulp additives.
(T4.3) and optimised use of waste lignin (T4.4). The main findings on this work package were: (1) cooking process (soda/synthraquinone or kraft) and kappa number of unbleached pulp (20 or 15) did not seem to have impact on mean area-weighted fibre length, mean fibre curl index, broken fibres content, fines content of the bleached pulps. On the other hand, vessels content was lower after Soda-AQ cooking than after Kraft cooking and a higher delignification of unbleached pulp (reduction of kappa number 20 to 15) induced (i) a reduction of mean fibre width, the hydrogen bonding potential,(ii) the increase in fibrillation of the bleached fibres and (iii) reduction of vessels content in the bleached pulp; (2) Soda-AQ process seemed to be the best process for the production of special pulp, due to reduction of vessels content in the pulp which could reduce speckles and picking problems during the printing of the paper; (3) it was more difficult to determine the best kappa number of the unbleached pulp because the reduction of fibre width, the hydrogen bonding potential should have a negative impact on mechanical properties of the final paper. However, the increase in fibrillation should improve mechanical properties and the reduction of vessels content should decrease speckles and picking problems; (4) E. globulus seems to be the best eucalyptus species for pulp manufacture because this pulp contained the longest fibres (this pulp should have the best mechanical properties) and fewer vessels (probably less speckles and picking problems); (5) after bleaching, morphological characteristics of elephant grass EG1 pulps (such as fibre length, relative bonded area index, fines content and vessels content) were equivalent for each pulp. On the other hand, the reduction of kappa number during cooking reduced the flexibility of the bleached fibres. Consequently, Soda-AQ process seemed as interesting as Kraft process for the production of elephant grass pulp; (6) Bleached pulp manufactured at kappa number 20 should have better mechanical properties than pulp at kappa number 15; (7) the bleaching of the pulp reduced the fibre length, broken fibres content, the hydrogen bonding potential, flexibility of the fibres, vessels content and increased fines content; (8) regarding of bleaching performance, among the pulps obtained from the Kraft and Soda-AQ processes, the Kraft process at kappa number 20 presented a best bleachability and the Soda-AQ process at kappa number 15 presented the worst; (9) the bleached pulp by Kraft process presented the higher values to viscosity, but the Soda-AQ process resulted in quite acceptable viscosity for IB (European E. globulus) and Elephant grass; (10) the Elephant grass pulp of kappa 15 showed the lowest bleachability and among the eucalypt the IB and hybrid DGxUGL showed the best bleachability; (11) family 5 and family 45 cellulases appear to provide the most significant strengthening benefits. Such improvements provide hope that the enzymes will provide similar benefit when added to deconstructed materials of inherently low papermaking value; (12) several enzymatic fiber modification trials have been conducted with the unconventionally deconstructed materials. Most of the deconstructed materials were readily modified by the cellulase treatments and the prepared handsheets showed improvement in tensile strength; (13) specifically the Cel45A and Cel17b endoglucanase families demonstrated the ability to improve resultant handsheet physical strength properties, the latter enzyme treatment without sacrificing tear strength. Also the Soda-AQ E. globulus fiber (both kappa 15 and 20) showed to be susceptible to enzymatic strengthening, resulting in tensile strengths equal to the otherwise stronger kraft fiber, thus enabling a completely sulphur-free pulping process. Refining trials carried out on the selected Soda-AQ E. Globulus pulps did not reveal any significant difference in the physical properties between the kappa 15 and 20 pulps, except in tear strength where the kappa 20 pulp was superior; (14) the enzymatic treatments of these pulps with both Cel45A and Cel17b revealed the need for further process optimization in order to obtain the expected benefits, although large increases in freeness developments were identified as well as increases around 10% in tensile strength after 100 PFI rev; (15) furthermore these unconventional pulps were investigated with regards to their susceptibility enzymatic delignification (i.e. bleaching) by the application of oxidoreductases both alone and in combination with mediators. These trials revealed that the high redox-potential laccase from Polyporus pinsitus showed synergistic behaviour when combined with violuric acid, reaching a brightness increase of 16 units for the kappa 20 pulp. Also interesting was it that the same absolute brightness values were obtained when kappa 15 and kappa 20 pulps were subjected to the enzymatic bleaching, which can be translated into increased pulp yield and cost savings on chemicals; (16) the data obtained throughout the studies which continuously favours the use of this unconventional sulphur-free pulp, especially at kappa 20, with regards to enzymatic strengthening and enzymatic bleaching; (17) the enzymatic delignification system was further optimised with the Coprinus cinereus peroxidase combined with violuric acid and it was shown that this system increased brightness by 7 units at a very low dosage of 0.3 mM of violuric acid and 0.5 mM of hydrogen peroxide; (18) it was also shown that conventional bleached kraft pulp with little effort could be converted into dissolving pulp of a proper grad via enzymatic routes. The application of xylanases and cellulase had positive influence on the solubilities (S10 and S18) and the intrinsic viscosities of the investigated pulps, thus enabling a
regular kraft mill to obtain dissolving pulp grades without the need for a pre-hydrolysis step; (19) the investigations of the biogas potential of the various substrates used in LIGNODECO revealed that the raw feedstock's themselves were rather recalcitrant and produced very little biogas, except for elephant grass which showed the highest yields; (20) when comparing the biogas yield of the kraft pulps to the raw feedstocks, it is clear that the deconstruction has had a positive impact on the digestibility; (21) the removal of a large part of the lignin fraction may also have a positive impact on the digestibility of the substrate; (22) when comparing the absolute values for the biogas yield of these deconstructed materials (580-690 Nmlg VS for the eucalyptus species) to the positive cellulose control (570 Nmlg VS) it is clear that the substrates are easily digested. However the higher biogas yield for the pulps may be a result of other volatile gasses (sulfur compounds) released from the pulps and may not represent methane and carbon dioxide arising from the anaerobic digestion of carbohydrates; (23) a minor contribution from the digestion of protein and fats in the feedstock is also included in the total biogas yield measured, but is considered to be negligible in the conversion calculation due to the small amounts present in the samples compared to the carbohydrate content; (24) there is no effect of the enzymes on the initial biogas yield; (25) with regards to both the conventional and unconventional deconstructed materials, all showed to be good substrates for biogas production, except the elephant grass which was inferior to the other tested substrates. However it is not advised to produce biogas from these perfectly nice pulped fibres, rather to used waste streams containing these and rejects for biogas production; (25) the investigations of the mill sludge effluent are still inconclusive giving the very short incubation time, which hardly gives off any right evaluation of the potential biogas potential; (27) As suggested on the basis of the chemical compositions (WP2), the shorter cooking times of 16-20h were sufficient for enzymatic hydrolysis of eucalyptus; (28) during 16-20h of LGF cooking, the fiber structure was opened up and delignified enough for efficient hydrolysis and highest proportion of the original feedstock could be released as sugars; (29) cooking time of 20h was thus selected for the pilot demonstration performed in WP5; (30) the alkaline extraction improves LGF pulp hydrolysability because of enhanced lignin and xylan removal; (31) the hydrolysis time of 48h was required for the maximum sugar release; (32) after LGF cooking and alkaline extraction, the DGxU2 hybrid gave highest sugar release as could be expected based on highest cellulose yield reported in WP2; (33) despite the relatively good cellulose yield and better delignification efficiency compared to the other eucalyptus clones, the E.globulus unexpectedly gave lowest sugar release; (34) the hydrolysis yield is to some extent dependent on cooking time, and optimal cooking time may vary between the clones. For E.globulus with lower initial lignin content the 20h cooking time may have been too long; (35) Alkaline oxidation (NaOH-O2) and soda-anthraquinone (Soda-AQ) treatments were tested as potential alkaline deconstruction methods for bioethanol production. The alkaline treatments were performed in Suzano for elephant grass (EG1) and G1xUGL eucalyptus hybrid. In all cases, pulps at kappa levels 50, 30 and 15 were prepared, and in all cases the G1xUGL eucalyptus provided higher ethanol yield than elephant grass; (36) cooking to low kappa level of 15 was necessary with both alkaline cooking methods (Soda-AQ, NaOH-O2) to provide reasonable ethanol production with EG; (37) for eucalyptus, the NaOH-O2 pretreatment was more suitable for bioethanol production than Soda-AQ. The Soda-AQ treatment required low kappa levels of 15, whereas after NaOH-O2 treatment kappa levels of 35-50 provided well hydrolysable pulp for fermentation. The NaOH-O2 treatment probably opens up the fiber ultrastructure better at the same lignin content, and even at high kappa levels the reject can be hydrolysed more efficiently; (38) the pressafiner had no significant mechanical crushing effect on EG. In previous lab scale experiments, the samples were ball milled, and higher ethanol yield was reported even for the reference without any enzymatic pretreatment; (39) the laccase treatment with HBT mediator (L HBT) clearly improved the enzymatic hydrolysability of elephant grass and E. globulus, and the effect was more pronounced for eucalyptus; (40) the laccase treatment without mediator (L) had practically no effect, and hydrolysability was equal with the control sample; (41) Probably due to the lower density and higher amorphicity, the elephant grass was much easier than E. globulus to hydrolyse even without any enzymatic pretreatment; (42) The actual bioethanol yields were well in line with the hydrolysability results; (43) the elephant grass provided better ethanol yield compared to E. globulus, and laccase with HBT mediator was better than laccase without any mediator; (44) the effect of HBT mediator was more pronounced with E. globulus; (45) In all cases evaluated, the ethanol yield was relatively low even though the samples were ball milled before the enzymatic treatments; (46) the ethanol production at pilot scale was very limited probably due to the insufficient biomass deconstruction and hydrolysis. The effect of ball milling on enzymatic hydrolysability was probably thus higher in previous lab scale experiments than the effect of laccase treatment; (47) in the studies of optimisation of enzymatic hydrolysis for the pilot pulp Optimal reducing sugar concentration after 48h of hydrolysis were found to be 9.5 g/l at an enzyme loading of 13.9 FPU/g_dm and 130.3
nkat/gdm for LGF pulp, 11.3 g/l sugars at 11.3 FPU/g_dm and 160 nkat/gdm for LGF-OH pulp and 10.1g/l at 10.7 FPU/g_dm and 259.7 nkat/gdm for Suzano pulp. Compared to the constant charges used in sample comparisons (10FPU Celluclast, 500 nkat/g Novozym 188), the optimum Celluclast dosage was slightly higher, but the Novozym charges could be reduced significantly; (48) for the surface model studies, the enzyme mixtures Celluclast and Novozym gave the highest variability in the response surface model. Xylanases only contributed minor variability. The Celluclast preparation already contains traces of xylanases activity, and the pulps contain only small amounts of xylan (below 10%), therefore the addition of purified xylanases has only little effect on the resulting sugar concentrations; (49) the LGF organosolv cooking with ethanol solvent and phosphinic acid catalyst produced well hydrolysable pulp with high ethanol yield, and the hydrolysability was further improved by alkaline extraction of the LGF fibers. Cooking time between 16-20h was optimal at relatively low cooking temperature of 130°C with 3.5% phosphinic acid and 15% water content; (50) in comparison with other deconstruction methods, the oxidative alkaline pretreatment (NaOH+O2) provided also well hydrolysable pulp at high kappa levels of 35-50, being another potential pretreatment method for bioethanol production from eucalyptus and elephant grass; (51) enzymatic laccase and hydrolytic pretreatments alone were not sufficient to open up fiber ultrastructure for efficient enzymatic hydrolysis, and thus ethanol fermentation; (52) regarding the potential of upgrading xylans as paper grade pulp additives, the results obtained were relevant to fine tune the methodology and assess the potential of the technology, positives result were obtained, such as: (a) the ideal conditions for xylans extraction are: 15 minutes, 15% of consistency and 400 kg/odt of NaOH; (b) Grass xylan deposition on wood pulp was successful, increasing the xylan content from 14.4 to 17.3%; (c) The deposited xylans are stable across bleaching and beating; (d) Bleaching yield gains of about 3% due to xylan deposition were achieved; (e) Pulps with improved strength properties and beatability were obtained; (f) The removal of the xylans from pulp using alkali treatment reduced significantly the chlorine demand in the bleaching sequence DPD at 90% ISO brightness; (g) the xylan depleted pulps derived from the xylan extraction treatment showed potential for production of special tissue grade papers with improved drainability, bulk, softness and water absorption capacity, and with acceptable tensile and tear strength. 5. WP5: Demonstration activities Work package 5 involved pilot plant demonstration trials on organosolve pulping, biomass enzymatic pretreatment, ECF bleaching and pulp beatability. Three different tasks were accomplished. The most promising LGF organosolve treatment was successfully scaled up in the VTT pilot plant unit (Task T5.1). The enzymatic pretreatment and pulping/bleaching pilot plant for eucalypt and elephant grass (Task 5.2) were successfully accomplished in the Centre Technique du Papier (CTP) in Grenoble, France. The eucalypt and elephant grass pulp beatability trials (Task T5.3) were also successfully performed at CTP. The main goal of Work Package 5 of the LIGNODECO project was the demonstration activities. The main findings were: (1) LGF organosolv process was performed using E. globulus since it was the best represents the Eucalyptus species available in Brazil and also in Europe; (2) the pilot demonstration resulted in LGF pulp with 56 % yield, and 51% total yield after alkaline extraction, which was somewhat lower than at lab scale, but also the delignification efficiency was better, probably due to the circulation of the liquor through chip pad throughout the cooking, and more efficient washing with hot liquors; (3) Cellulose yield of LGF pilot scale was comparable to the laboratory pulp; (4) Despite the similar cellulose content, the hydrolysability of pilot pulp was significantly better compared to the corresponding lab pulp, or any of the LGF pulps produced at lab scale (Suzano/DGxU2). This is probably due to the more efficient delignification and also lower xylan content; (5) After alkaline extraction, the maximum hydrolysis yield was reached already after 24h hydrolysis; (6) in comparison with the alkaline oxidation pretreatment, the LGF organosolv cooking at pilot scale produced slightly better bioethanol yield; (7) After alkaline extraction of LGF pulp, clearly higher bioethanol production was shown compared to the Soda-O2 pulp; (8) The better bioethanol yield of LGF pulp was in line with higher cellulose and lower xylan content compared to Soda-O2 pulp; (9) The LGF organosolv cooking was successfully demonstrated also at pilot scale, producing well hydrolysable biomass with high ethanol yield; (10) nearly 90% of the theoretical ethanol yield was reached after the alkaline extraction of LGF pulp, this was higher compared to the alkaline oxidation, which also produced well hydrolysable pulp for bioethanol production; (11) as obtained in WP4, the bioethanol yield after enzymatic treatments at pilot scale was very limited; (12) the enzymatic deconstruction using hydrolyses, percentage of cellulose was slightly lower in the pulp with cellulase application than in the control pulp; (13) two pulps, one treated with hydrolyses and other control were sent to VTT in order to perform saccharification and fermentation for the production of bio-ethanol, however, according to results obtained by VTT, the enzymatic treatment was less efficient at pilot scale than in laboratory; (14) the enzymatic deconstruction using oxido-reductases for deconstruction of E. globulus after defibering step contained higher fine elements and fibrillated fibres
than refined pulp (pulp before treatment), however, the comparison between the different treated pulps was difficult and MorFi analysis did not show difference between these pulps; (15) regarding the chemical deconstruction using alkaline processes The reduction of active alkali (17.5 versus 15.5) and the increase in L/W ratio allowed to obtain similar results as Suzano ones in lab scale; (16) for Soda-AQ process after pilot trial, kappa number of the elephant grass pulp (17) was lower than pulp produced in laboratory (kappa number: 20) and this problem came from the raw material, due to a part of the raw material was contaminated by fungi; (18) brightness of Soda-AQ pulp from pilot plant was higher than laboratorial Kraft pulp, maybe due to the lower formation of cathecol groups, however, polysaccharides seemed more degraded during Soda/Anthraquinone process; (19) Soda-AQ and Kraft pulps had similar morphological fibres characteristics, except for curl index and vessels content; (20) Soda/Anthraquinone had lower vessels content; (21) Soda-AQ pulp seemed slightly more flexible than Kraft fibre (flexible fibre will develop better the strength properties of the final paper); (22) for the pulp bleached in pilot scale the brightness of the pulps and morphological fibres characteristic were similar after bleaching sequence, except curl index; (23) after bleaching, Kraft pulp fibres seemed slightly more flexible than Soda/Anthraquinone pulp fibres; (24) opacity of pulp produced with Kraft process was lower than Soda-AQ pulp one; (25) for viscosity measurement, trend observed in unbleached pulp was confirmed in bleached pulp. Soda-AQ process degraded more the polysaccharides than Kraft cooking, inducing lower mechanical properties of the final paper; (26) Regarding the bleached Soda-AQ and Kraft pulps were refined at pilot scale and the main findings were: (a) For similar energy consumption, the best mechanical properties were obtained with 0.2 Ws/m refining intensity, because the eucalyptus chemical pulp fibres were quite amenable to fibrillation and therefore to refining; (b) Soda-AQ pulp consumed less energy than Kraft pulp to reach a given drainage index however, mechanical properties of Soda-AQ pulp were lower than these of the Kraft pulp; (c) the negative results with xylans addition on mechanical properties could be attributed to their drying for sending from UFV to CTP, since in preliminary studies done by UFV, prove that the addition of xylans in Soda-AQ pulp allowed to improve mechanical properties and to compensate this difference; (27) alkaline demonstration trial at pilot scale validated results obtained at laboratory; (28) Soda-AQ pulp could replace Kraft pulp, since it is a sulfur-free process which is interesting for limiting air pollution, however, soda/anthraquinone pulp had lower mechanical properties than Kraft pulp; (30) the enzymatic deconstructions using oxido-reductases or hydrolases performed in laboratory were also validated at pilot scale.

Potential Impact:

Potential impact (including the socio-economic impact and the wider societal implications of the project so far)

With the accomplishment of LIGNODECO work packages and tasks, including the pilot trials, the knowledge about eucalypt and elephant grass feedstocks as well as their applications in biorefinery for production of specialty pulps, biofuels and bio-products, was certainly raised to a much higher level.

The LIGNODECO research activities focused on solutions responding to RTD needs identified in especially the European Forest-based Sector Technology Platform (www.forestplatform.org) but also in Sustainable Chemistry Technology Platform (www.suschem.org). The optimal and sustainable use of wood and forest industry side-streams as feedstocks for biorefineries has been strongly emphasised in strategic research agenda of the European FTP. Potential synergy between traditional forest-based refining chains with other industries including chemical industry and energy sector has been emphasized, especially in the case of production of high value chemicals, polymers and fuels from forest raw materials. The SusChem Strategic Research Agenda (SRA) has defined biocatalysis and Biorefinery as key objectives and LIGNODECO readyly answers this need.

The world economy is tied so closely to petroleum products and oil imports that disruption in oil supplies can result in severe economic and social impacts. Conventional oil production will peak in the near future, and the resulting energy transition requires a portfolio of responses, including unconventional fossil resources and biofuels. Environmental quality and climate change due to energy emissions are additional concerns. Biofuels, especially cellulosic ethanol, constitute the only renewable liquid transportation fuel option that can be integrated readily with petroleum-based fuels, fleets, and infrastructure. Production and use of biofuels can provide substantial benefits to national energy security, economic growth, and environmental quality. About 60% of known oil reserves are found in sensitive and volatile regions of the globe. Increasing strain on World oil supply is expected as developing countries become more industrialized and use more energy. Any strategy to reduce reliance on imported oil will involve a mix of energy technologies including conservation. Biofuels are an attractive option to be part of that mix because biomass is a domestic, secure, and abundant feedstock. Global availability of biomass feedstocks also would provide an international alternative to dependence on an increasingly strained oil-distribution system as
well as a ready market for biofuel-production technologies. The expected impact of LIGNODECO is to decrease the Europe's dependency on fossil raw materials for production of chemicals and energy and to enable European industry to double its revenues within next 10 years by more efficient raw material exploitation and valorisation to special papers and biofuels.

Although Brazilian position in regard to oil is currently comfortable since over 40% of our energy matrix comes from renewable sources, our efforts on producing biofuels shall continue.

Significant European emphasis is currently being put into maximized exploitation of different types of biomass sources for sustainable production of value added products, which could replace petroleum based products. The main drivers behind this development, are i) the climate change, ii) the price and dependency on imported crude oil, both of these impacting under the auspices of an exponentially increasing global population. An expected outcome is new forest biomass-based processing concepts that will result in highly efficient use of the original biomass feedstock. If taken into use, these concepts will build bridges between forest and the processing industries. These concepts would also generate more economic value, ethically more clever use of our biomass resources and land, and environmental benefits.

The strong partnership between the European and Brazilian research groups ascertained that the most relevant expertise in both environments was combined for mutual benefit. On a general level, the technologies developed within LIGNODECO improved the competitiveness of the pulp and paper and processing industries as well as the forestry sector supporting these sectors by securing jobs and investments. At least three technologies developed within LIGNODECO (two patented and one underway) represent significant steps towards transformation of traditional pulp and paper industry, which has been challenged by low-cost competition, to a new business era by creating additional markets for paper. They include processes for production of special high yield printing and writing paper grade pulp, high strength eucalypt kraft pulp, and highly absorbent tissue grade pulp. Additionally, it was developed a process that permits cross-sectorial applications in the biofuel industry, namely the LGF organosolve pretreatment method for bioethanol production. These will help increasing the revenues obtained by the pulp and paper industry through new processes, added value products and new business models. It also fosters scale-intensive and specialised suppliers industry through the adoption and integration of new advanced technologies.

Consequently, LIGNODECO contributed to the European Research Area as well as to the development of Brazilian RTD activities in the field of sustainable development based on the production of biomass-based products and fuels, an area where Brazil occupies a world-leader position because of its large availability of natural areas for fast growing forest and agricultural lignocellulosic crops.

Furthermore, LIGNODECO addresses several EU-level policies. LIGNODECO contributes directly to the European Life Sciences and Biotechnology Strategy and Action Plan (2002-2010), and thus the “Lisbon objectives”. The European Union has launched a series of policy initiatives to commit EU to the Kyoto Protocol of reducing greenhouse gas emissions (COM (2005)35), to use bioresources leading to more sustainable production processes (COM 2005 670; 6th Environmental Action Program), to reach 20% renewable products and energy by 2020 (Renewable Energy Roadmap COM 2006 848; Biomass Action Plan COM (2005) 628; Environmental Technology action Plan ETAP COM (2004) 38, SET COM (2006) 847) and to boost the production and use of biomass-based materials (Lead Market Initiative COM(2007)860). LIGNODECO contributed directly to these policy initiatives by creating alternative wood derived products to petroleum-based products or by developing new product concepts from the forest side-streams and by developing technologies for maximized and value-added raw material utilization in forest to mill value chain. Finally, LIGNODECO contributed in different extents to the implementation and evolution of the following EU policies: (1) Industrial policy and employment is affected by improved competitiveness of European pulp and paper industry as added-value is generated to the current process side-streams. Also new business opportunities are developed for SMEs focusing on by-product valorisation and end-use. The employment is positively affected as the competitiveness of European chemical, forestry, material and enzyme industry is improved by generation of novel products and processes. (2) EU forestry strategy is positively affected by developing technologies for full exploitation of wood raw materials to value-added components. Thus, the economic opportunities to forest owners are enhanced and the competitiveness of forestry management is improved. (3) Environment and sustainable development is affected by developing sustainable and targeted processes for valorisation of process side-streams of the forestry as well as pulp and paper processing industries and thereby minimizing their environmental impact. A pulp mill implementing the most recently available technologies for biorefining of its side-streams has a significant impact on environmental issues. (4) European cohesion is improved by the multinational approach and by incorporation of industrial partners representing different aspects of the value chain. Development and
implementation of advanced technologies and new methodologies requires the joint efforts of selected experts with complementary skills: pulping experts, biotechnologists, process engineers and analytical chemists. Best expertise in these disciplines can be found in Brazilian-European consortia. (5) Rural development is positively affected. Pulp mills are usually located in rural and sparsely populated areas. A co-location with new Biorefinery industries or concepts would mean employment opportunities for the local communities in areas with depopulation and decreasing job opportunities. The specific impacts of the LIGNODECO regarding scientific and technical, industrial and economic, and environmental and social were significant.

In LIGNODECO WP1 it was possible to identify the most suitable Brazilian feedstocks, among the various eucalypt and elephant grass raw materials, that are adapted to production of special grade pulps, biofuels and bio-products. Different and unique wood clones from the nationwide Brazilian Genolyptus program were selected for the desired product manufacture. In addition, the potential of elephant grass to make paper pulp, biofuels and bio-products was unveiled. Furthermore, it was relevant to understand the large variations that exist among different feedstocks, the adaptation of analytical tools for evaluation of a large variety of untreated and pretreated biomasses. All these developments may have significant impact on the forestry and agroindustry in general in the future.

In LIGNODECO WP2 the main scientific and technical accomplishments included the selection among the six biomass deconstruction methods, the ones more adapted to each feedstock. It was developed and patented a organosolve pretreatment method, assisted with phosphinic acid, that resulted in high biomass glucose-release for bioethanol production. A high yield non-sulfur alkaline deconstruction process adapted to Eucalyptus globulus wood containing lignin of high syringyl/guaiacyl ratio was disclosed. A medium sugar release bio-mechanical pretreatment for bioethanol production was developed and demonstrated in pilot trials. It was established that the soda/O2 and the organosolve deconstruction methods are the most suitable for bioethanol production. It was identified situations where that Kraft process may be replaced by the Soda/AQ, with significant environmental gains. It was also determined the importance of mechanical treatments prior to enzymatic assisted deconstruction methods and the challenges associated with this technique.

Brand new analytical tools were developed and tested in LIGNODECO WP3 for unusual feedstocks, including imunolabelling to quantify hemiceluloses in the fibre surface that were deposited by our patented technology, and Py-GC/MS, TMAH, HSQC, DFRC to evaluate the unusual elephant grass lignins. The adaptation of these tools for the less understood Brazilian feedstocks is quite relevant for the pulp and paper and related industries.

In WP4 it was developed a process for production of high yield (+6%) and high quality printing and writing paper grade pulp by deposition of xylans onto eucalypt Kraft pulp in the oxygen delignification stage (patented), a process for production of a high strength eucalyptus kraft pulp through fiber treatment with a specially designed cellulase blend (patented), a process for production highly absorbent tissue paper grade (patenting underway) and a biogas production method based on pulp mill effluent sludge.

An important finding of LIGNODECO was that the elephant grass material makes a very good tissue paper, after deconstruction via de soda/AQ process. The left over bits from elephant grass finds good application in the bioethanol industry (xylans) and energy industry (lignin).

In summary, the main scientific and technical impacts achieved within LIGNODECO umbrella were: (1) the selection of appropriate biomass for conversion of pulp mills into biorefineries, (2) optimisation of alkaline and organosolve pretreatments for deconstruction of such biomass with the view of producing paper pulp but also other bio-products of high added value, (3) development of wild-type and improved enzymes for biomass pretreatments (4) scaled up process for physical/ enzymatic deconstruction, (5) advances in analytical methods applied to eucalypt and elephant grass feedstocks, (6) development a high yield, high beatability, and high tensile strength printing and writing paper pulp grade from eucalypt, (7) development of a highly absorbent tissue grade pulp, (8) development of techniques to produce biogas from kraft mill effluent sludge. Most of the technical and scientific developments aforementioned that resulted in pulp and paper mills increased efficiency, with the generation of novel products form woody and nonwoody biomass are particularly relevant for the environment. Anytime products derived from fossil fuels are replaced by biomass based ones, great environmental gains are achieve. In our particular case, the highly absorbent and high strength paper products developed minimize or eliminate the use of fossil based additives in the papermaking process. In addition, the organosolve pretreatment process developed for production of biofuel is also environmentally friendly since it uses ethanol as the solvent, the same ethanol bio-product made from the
biomass. When fossil fuels are consumed, carbon sequestered from the global carbon cycle for millions of years is released into the atmosphere, where it accumulates. Biofuel consumption can release considerably less CO2, depending on how it is produced. The photosynthetic production of new generations of biomass takes up the CO2 released from biofuel production and use. Over time a mature bioenergy economy will substitute biomass-derived energy sources for fossil fuel, further reducing net emissions.

Perennial grasses and other bioenergy crops have many significant environmental benefits over traditional row crops. Perennial energy crops provide a better environment for more-diverse wildlife habitation. Their extensive root systems increase nutrient capture, improve soil quality, sequester carbon, and reduce erosion. Ethanol, when used as a transportation fuel, emits less sulphur, carbon monoxide, particulates and greenhouse gases.

The societal implication of the technologies developed under the LIGNODECO project involves two major aspects: (1) the benefits to society in terms of well-being and (2) the capacitation of personnel. In the first case, the high quality printing and writing and tissue paper grades developed in the project will improve the quality of life of many users not only in terms of customer satisfaction but also cost wise. In the latter case, LIGNIDECO was quite successful with the capacitation of a large number of students with BS, MS and DS degrees, Postdocs, technicians, etc. Only at UFV the number of students trained exceeds twenty. The special training on characterization of biomass, biomass deconstruction and biomass utilization is quite useful in the forthcoming bio-economy. The pulp and paper, biofuel and bio-products industry is already benefitting from LIGNODECO trained personnel. Only at UFV about 10 students that worked in the LIGNODECO project were hired in the last two years to serve the forest, pulp and paper and sugar cane/ethanol industry.

Gender issues were carefully considered during the development of the LIGNODECO project. In general the female: male ratios for VTT, CIB, IRNAS, Novozymes, UFV, Suzano and CTP were, respectively: 6/3, 4/3, 3/2, 2/3, 1/1, 1/1 and 5/4. Gender equality was strongly supported within the LIGNODECO project. All recruitment during the course of the project aimed for a 1:1 Female: Male ratio. Of course the qualifications also played a role in the selection of personnel and not always it was possible to meet the even ratio. The management of the project always aimed to maintain equal numbers of both genders throughout the project lifetime. Advertisements were checked to ensure absence of gender bias and the aim was to appoint equal numbers of new staff to the project. This is in agreement with COM (99) 76 final which advocates “at least 40% participation of women at all levels in implementing and managing research projects”. Recruitment procedures conformed to national and European standards. The consortium ensured that all advertisements gave equal opportunities and that no gender discrimination occurred in the allocation of its funds. The partners and management always promoted the participation of women throughout the project.

Dissemination of foreground
The results from LIGNODECO were disseminated following EC policies. The main scientific results were published in specialized and internationally recognized scientific journals and presented at different National and International Scientific Conferences. The matters concerning dissemination and Use of Foreground was clarified in the Consortium Agreement (see section 2.1) signed by all partners. Specific cases were discussed within the technical committee. The Consortium granted preferential rights to the industrial Partners in exploiting Foreground. If none of the LIGNODECO industrial partners were interested in exploiting Foreground, a search for third parties interested in the project was performed by the partners.

During the course of the three year LIGNODECO project, there were two patent applications and there is another one in preparation (production of highly absorbent tissue paper). The first application was registered in the Brazilian National Institute for Intellectual Property (INPI) under number 014110000228 with the title “Processo de Deposição de Xilanas Durante o Pré-branqueamento de Polpa Celulósica”. The second patent application has been prepared and is in the process of being filled in the European Patent Office with the title “Improved drainage of paper pulp”. As of this moment, there have been 13 peer-reviewed publications and 44 oral/visual presentations in conferences, workshops, seminars, colloquia, etc. It is anticipated that there will be at least 10 more peer-reviewed publications and 20-25 oral/visual presentations in conferences. This estimation is reasonable considering that a lot of the more significant project results were obtained in the last six months with the conclusion of the tasks on WP4. Some important upcoming events that will attend heavily by LIGNODECO participants are: 17th International Symposium on Wood, Fibre and Pulping Chemistry, that will be held in Vancouver, Canada, during June 12-
14, 2013; the 6th International Colloquium on Eucalyptus Pulp, that will be held in Colonia del Sacramento, Uruguay, during November 24-27, 2013; and the 13th European Workshop on Lignocellulosics and Pulp, that will be held in Seville, Spain, during June 24-27, 2014.

A significant number of MS and Ph.D students were trained under LIGNODECO’s umbrella. To this date 7 Master Thesis and 2 Ph.D Dissertation. These students disseminated knowledge in their work places and communities, in addition to the publication of their dissertations and thesis. Also post-doctorate students and college professors disseminated the LIGNODECO activities and knowledge among their peers and students. The three most prominent Ph.D thesis under development in LIGNODECO are: (1) study of the chemical composition of fast growing lignocellulosic crops and modification of their lignins during alkaline deconstruction” by Pepijn Prinsen (University of Seville), (2) optimization of laccase-based pretreatments for the enzymatic deconstruction of woody and nonwoody lignocellulosic feedstocks” by Alejandro Rico (University of Seville), (3) the biorefinery concept applied to pulp and paper industry” by Fernando José Borges Gomes (Federal University of Viçosa).

A web-page was set up and maintained during the three years of the LIGNODECO project (http://www.lignodeco.com.br). This webpage will be maintained at least one more year after project completion. Its content includes a summary for public dissemination, list of publications and patents, and information on other dissemination activities. An intranet section was organized at the web-site to facilitate the direct exchange of documents and other information between partners, such as technical reports, project documents, publications, minutes of meetings, etc. In addition to the use of web-page and participation in meetings, a permanent contact between partners was maintained by teleconferencing and electronic mail. Three dissemination booklets were developed to specifically disseminate LIGNODECO, in which the cutting edge scientific developments were described in vulgarized terms so that less trained personnel within communities and private enterprises can convert top notch scientific knowledge into practice. Such booklets were prepared in the beginning of the project, at 18 month along with the first periodic report and at 36 month along with the final report.

The LIGNODECO project results to the public and private sectors were also disseminated through two different conferences, namely: (1) 5th International Colloquium on Eucalyptus Pulp, (2) The V Pulp and Paper Seminar: Lignin Biorefinery – From Biomass to Product. These two events run every other year in South America in alternate fashion. The 5th Colloquium was created in the year of 2003, and has become an important forum for discussions on production and utilization of eucalyptus. The 5th ICEP was held in Porto Seguro, Bahia, Brazil, in May 9-12, 2011 and focused on the integration of eucalyptus pulp mills with liquid and solid fuel production, materials and chemicals units. In other words, the vision of the eucalyptus Kraft mill functioning as bio-refinery was thoroughly discussed in this important event that gathered 287 people. The V Seminar was created in 2004 and is an important forum for dissemination of the new developments in the pulp and paper industry. The V Pulp and Paper Seminar was held in Viçosa, Minas Gerais, Brazil, in September 13-14, 2012 and focused on lignin biorefinery (from biomass to product) with the participation of 197 delegates.

Pamphlets about the LIGNODECO project, including a project summary, main goals, deliverables, milestones, etc. were prepared by the coordination and distributed to the pertinent public, in conferences, seminars, workshops and other public gatherings, in addition to electronic distribution (internet). Press releases on an annual basis were done on the main findings of the project, through important communication vehicles such as Tappi, ABTCP, Bracelpa, SPCI, PI, Cellulose online, ATIP, ATCP-Chile Technicelpa, etc.

The involvement of local politicians to bring attention of LIGNODECO to the communities is was carried through the Secretary for Science and Technology of Minas Gerais State. Suzano and Novozymes corporations have disseminated the LIGNODECO development project within there are of influence.

Exploitation of the Foreground

The technologies developed under LIGNODECO will be exploited by the two industrial partners (Suzano and Novozymes) and/or licensed to third parties if considered pertinent. The main products developed were: (1) high throughput selected eucalyptus and elephant grass biomass material suitable for biofuel and bio-products industry, (2) novel cellulase enzyme for improving paper strength, (3) a high yield and high quality printing and writing paper grade pulp, (4) a highly absorbent tissue grade paper pulp, and (5) a high glucose release biomass pretreatment with solvents catalysed with phosphinic acid. These new processes and products will be exploited by forestry companies and small farmers working in cooperatives (product 1), enzyme producing companies (product 2), pulp and paper companies (products 3 and 4), biofuel/energy and pulp and paper
companies (product 5). The beneficiaries of these new products will be the society as whole and obviously the suppliers to the industries that will exploit the technologies created.

Some of the findings of WP2 and WP4 were confirmed on pilot scale trials and raised interest from the two LIGNODECO industrial partners. These partners are world-leading companies in the fields of enzymes and pulp and paper production. Suzano pulp company is interested in scaling up the high yield (+6%) and high quality printing and writing paper grade pulp and the highly absorbent tissue paper grade pulp processes in one of their pulp production units in Limeira-SP. On the other hand, Novozymes is searching for a client in order to establish a mill trial for application of their special cellulase blend that improves paper strength. After the importance of the lignin syringyl/guaiacyl ratio for any deconstruction method was established, Suzano Forest Company has been dedicating great attention to selecting wood having such trait in their tree improvement programs.

List of Websites:
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