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(54) Title: INTEGRATED PROCESS FOR THE SELECTIVE FRACTIONATION AND SEPARATION OF LIGNOCELLULOSE IN ITS MAIN COMPONENTS

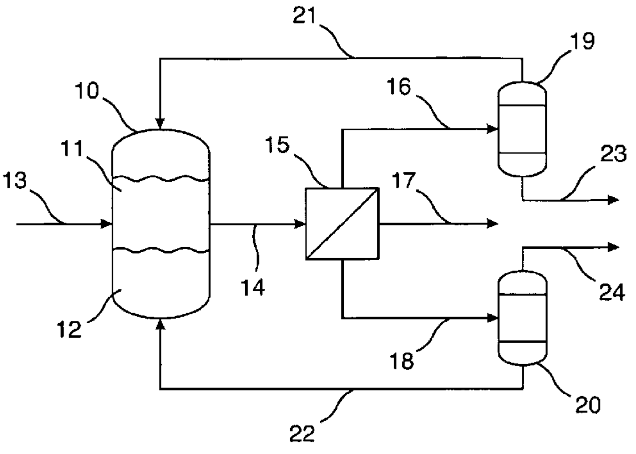

FIG. 1

(57) Abstract: A method for processing lignocellulose comprises the step of contacting a lignocellulosic substrate with a biphasic liquid system in a reaction vessel, the biphasic liquid system comprising a first, aqueous phase and a second, organic phase which is at least partially immiscible with the first, aqueous phase under the conditions under which step a) is performed. The first, aqueous phase further comprises a carboxylic acid catalyst and the second, organic phase comprises a solvent selected from the group comprising esters, lactones, ethers and/or cyclic ethers with the proviso that said solvent does not comprise a hydroxyl group or hydroxyl groups. Preferred are oxalic acid as a catalyst and 2-methyltetrahydrofuran as the organic solvent. The set-up of a two-phase system affords the selective hemicellulose hydrolysis, together with efficient in situ cellulose-pulp delignification and in situ lignin extraction. Overall, all components are easily separated and are ready for further bio-refinery processing.

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Integrated process for the selective fractionation and separation of lignocellulose in its main components

The present invention relates to a method for processing lignocellulose, comprising the step of contacting a lignocellulosic substrate with a biphasic liquid system.

Within the bio-based economy, competitive routes for the efficient (ligno)cellulose processing need to be developed. Lignocellulose (in particular, but not restricted to, "wood") is mainly composed of three components: lignin, hemicellulose (composed of sugars: principally xylose, galactose, glucose), and cellulose (composed of glucose). A selective lignocellulose fractionation into these three components may lead to a number of useful chemicals and commodities. These compounds can be used as such, or conversely can be employed via further modifications, (bio)chemical derivatizations, etc.

For example, lignin can be used in the fragrance or food industry, as a thermoplastic, as a source of phenolic derivatives, or directly as a biofuel. From hemicellulose sugars, xylose provides xylitol and furfural. Furthermore, it can be used by microorganisms able to metabolize C_5 sugars to produce fuels and other bio-based commodities. Finally, cellulose pulps are used in pulp & paper industries, as well as a source of C_6 fermentable sugars (to produce fuels or chemical building blocks).

Several methods have been reported for lignocellulose processing and fractionation. Amongst them, an important strategy is the so-called "organosolv" process (review article: Johansson et al., *Biomass* 1987, 13, 45-65). Said process involves the acidic treatment of lignocellulose at high temperatures and pressures in aqueous systems in combinations with a cosolvent (typically, e.g. ethanol, methanol, acetone, etc), affording a (more or less degraded) lignin, some cellulose pulp and an aqueous solutions containing xylose and glucose.

Although it appears promising, the organosolv processes has several drawbacks. For instance, in most of the conditions tested, the technology is largely unspecific, leading to corrosion or formation of by-products and further degradation of produced sugars (to compounds like furfural, hydroxymethylfurfural (HMF), levulinic acid, etc). Moreover, the frequently required use of strong inorganic acids (e.g., sulphuric acid) necessitates a neutralization (e.g., addition of base) before further treatments. This usually leads to a significant formation of wastes (e.g., gypsum), thus adversely influencing the environmental and economic outcome of the intended route. Finally, most of the organic solvents applied are alcohols (e.g., ethanol, methanol). It has been reported that by high temperatures and at acidic conditions, such alcohols lead to etherification, thus leading to solvent losses, hampering its recyclability, and therefore compromising the delicate economics of the process (*ChemSusChem*, 2010, 3, 1349).

Generally, the cosolvents employed in the organosolv processes are miscible with water at least during the conditions under which the extraction is performed. Thus, to efficiently remove lignin from pulp fibers, strong conditions of pressure and temperature must be applied, leading again to degradation problems. Furthermore, alcoholic co-solvents have the disadvantage that etherification and by-product formation might happen under the acidic conditions of an organosolv process (*ChemSusChem*, 2010, 3, 1349).

Conclusively, the use of inert and bio-based, water-non-miscible solvents may represent an interesting approach for an efficient and in situ lignocellulose fractionation. As an example of an inert solvent, Tetrahydrofuran (THF) has been used in some organosolv processes (Johansson et al., *Biomass* 1987, 13, 45-65). Yet with THF, being miscible with water, problems of delignification may be expected under mild reaction conditions.

In this respect, US 5,730,837 discloses a method for separating lignocellulosic material into (a) lignin, (b) cellulose, and (c) hemicellulose and dissolved sugars. Wood or herbaceous biomass is digested at elevated temperatures in a single-phase mixture of alcohol, water and a water-immiscible organic solvent (e.g., a ketone). After digestion, the amount of water or organic solvent is adjusted so that there is a phase separation. The lignin is present in the organic solvent, the cellulose is present in a solid pulp phase, and the aqueous phase includes hemicellulose and any dissolved sugars.

WO 1995/18260 A1 is concerned with a method of delignifying wood or plant material, both virgin and post-consumer with a recyclable liquid composition comprising sodium silicate up to 2.5%, sodium hydroxide up to 2.5%, alkyl or dialkyl glycol ether and/or diglycol ether and/or propylene glycol ether in an amount of 10 to 40%, hydrogen peroxide up to 10%, triethylamine and/or diethylmethylamine and/or dimethyl pyridine and/or methyl pyridine and/or methyl piperidine up to 10% and water to give 100%. The liquid composition has a lower critical solution temperature between about 0 °C and about 100 °C and the method comprises heating said wood or plant material with an effective amount of said liquid composition to release lignin from said wood or plant material.

The above-mentioned international patent application also discloses a method of delignifying a lignin-containing plant-derived material with a recyclable liquid composition comprising water and an organic compound which can be azeotropically purified, said liquid composition having a lower critical solution temperature of between 0 °C and 100 °C above which lower critical solution temperature said liquid composition divides into a heavier aqueous phase and a lighter organic phase. This method comprises treating said lignin-containing plant derived material with a lignin-removing amount of said liquid composition at a temperature between 100 °C and 210 °C for sufficient time to release lignin from said lignin containing plant-derived material.

Regarding acidic conditions it is known that some organic dicarboxylic acids (e.g., oxalic, maleic, fumaric, etc.) have been used for (ligno)cellulose depolymerization, as stated in: J.W. Lee et al., *Biores. Technol.* 2010, 100, 6307-6311, A.M. Kootstra et al., *Biotech. Biofuels*, 2009, DOI: 10.1186/1754-6834-2-31; *Biochem. Eng. J.* 2009, 46, 126; *Biochem. Eng. J.* 2009, 43, 92; J.P.M. Sanders et al., WO 2009/145617 A2; Y. Lu et al., *Biotechnol. Prog.*, 2007, 23, 116; S. Meng et al., CN 101199944; N.S. Mosier et al., *Biotech. Bioeng.*, 2004, 86, 756; *Biotech. Bioeng.*, 2002, 79, 610; *Biotechnol. Prog.*, 2001, 17, 474; Paszner et al., US 4,409,032.

This route may open important options for a complete biomass valorization by fractionating the lignocellulosic residues in a controlled and rational way. As drawback, however, to efficiently process crystalline and packed celluloses via this route, again high temperatures (> 160 °C) must be applied. When these dicarboxylic acids are used under those conditions, a significant degradation of sugars (to form furfural, hydroxymethylfurfural, etc) is observed. Interestingly, at significantly milder conditions (e.g., < 130 °C) dicarboxylic acids are able to efficiently hydrolyze hemicellulose, which is a polysaccharide that is less crystalline and packed than pure cellulose. Thus, the application of such acids at ca. 100 – 130 °C have found use in the pulp & paper industry.

Herein, to get proper, large cellulose fibers for paper industry (the so called “pulp”), it is important to selectively remove lignin and hemicelluloses. Therefore, the treatment of lignocellulosic materials with oxalic acid, at 100 – 130 °C leads to the selective hydrolysis of hemicellulose, thus awarding cellulose fibers somewhat purified and prepared for pulp & paper issues (M. Akhtar et al., WO 2007/008689 A2; WO 2002/075043 A1). Furthermore, this approach has also been disclosed for the xylose production (again from selective hemicellulose hydrolysis), by means of oxalic acid hydrolysis at 100 °C, and further crystallization of xylose (Steiner et al., US 3,586,537).

In said processes a considerable amount of lignin remains in the cellulose pulp, and thus bleaching and further treatments of the fibers are needed, e.g., using bisulfite (M. Akhtar et al., WO 2002/075043 A1), leading to waste generation.

Hence there is still a need in the art for an efficient and mild method that may allow the proper in situ lignocellulose fractionation into its main three components of hemicellulose sugars, lignin, and cellulose pulp, without degrading them, to allow their further (bio)chemical valorization. Furthermore, in view of the depletion of petroleum resources, said method should preferably only employ bio-based resources, thus assuring the sustainability of the method in the coming decades. Finally, such a method should be designed to be as waste-free as possible, including the recycling of all components (e.g., catalyst) employed within. None of the presently reported methods for lignocellulose fractionation can address all these important issues simultaneously. The present invention therefore has the object of providing such a method.

This object is achieved in the present invention by a method for processing lignocellulose, comprising the step of:

a) contacting a lignocellulosic substrate with a biphasic liquid system in a reaction vessel,

the biphasic liquid system comprising a first, aqueous phase and a second, organic phase which is at least partially immiscible with the first, aqueous phase under the conditions under which step a) is performed,

wherein the first, aqueous phase further comprises a carboxylic acid catalyst and

wherein the second, organic phase comprises a solvent selected from the group comprising esters, lactones, ethers and/or cyclic ethers, with the proviso that said solvent does not comprise a hydroxyl group or hydroxyl groups.

The method according to the invention provides a novel approach for selective lignocellulose fractionation. Carboxylic acid catalysts depolymerize hemicellulose selectively with the possibility of mild conditions being employed, whereas cellulose-pulp is not hydrolyzed. Surprisingly, it has been found that the set-up of a two-phase system affords the selective hemicellulose hydrolysis, together with efficient in situ cellulose-pulp delignification and in situ lignin extraction into the organic phase. Water-soluble sugars remain in the aqueous phase and may be collected for further processing. Insoluble cellulose-pulp is then accessible for subsequent efficient enzymatic hydrolysis to afford glucose.

Overall, all components are easily separated and are ready for further bio-refinery processing. No significant by-products or degradations are observed due to the mild conditions which may be applied. Moreover, the reagents and solvents used can be derived from bio-based resources. As organic solvent, for example bio-based 2-methyltetrahydrofuran (2-MTHF) may be used, showing excellent properties for lignin extraction, as well as for delignification of cellulose pulp fibers.

The lignocellulosic substrate (which may also be referred to as lignocellulosic feedstock) used may be any lignocellulose containing biomass such as wood or pre-processed lignocellulose. Examples include, but are not limited to, non-woody plant biomass, cultivated crops such as, but not limited to grasses, for example, but not limited to, C4 grasses, such as switch grass, cord grass, rye grass, miscanthus, reed canary grass, or a combination thereof, sugar processing residues, for example, but not limited to, baggase, beet pulp, or a combination thereof, agricultural residues, for example, but not limited to, soybean stover, corn stover, rice straw, rice hulls, barley straw, corn cobs, wheat straw, canola straw, oat straw, oat hulls, corn fiber, or a combination thereof, forestry biomass for example, but not limited to, recycled wood pulp fiber, sawdust, hardwood, for example aspen wood, softwood, for example birch wood, fir wood or a combination thereof. Furthermore, the lignocellulosic substrate may comprise cellulosic waste material or forestry waste materials such as, but not limited to, newsprint, cardboard and the like. The lignocellulosic substrate may comprise one species of fiber or, alternatively, it may comprise a mixture of fibers that originate from different lignocellulosic feedstocks. In addition, the lignocellulosic substrate may comprise fresh lignocellulosic feedstock, partially dried lignocellulosic feedstock, fully dried lignocellulosic feedstock, or a combination thereof.

The reaction vessel for step a) may be a batch reactor or a continuous reactor. Hence, the method according to the invention may be performed as a batch or a continuous process. If desired, the reaction vessel may be equipped with stirrers, solvent reflux or pressurizing installations.

In the method according to the invention the lignocellulosic substrate is contacted with a biphasic liquid system. This is to be understood as the liquid system having two distinct liquid phases under the conditions of the method according to the invention. The initial lignocellulosic substrate is present as a third, solid phase. Other solid phases such as heterogenous catalysts, molecular sieves, ion exchangers, solid-state acids and the like are also possible. Likewise, a gas phase, for example for pressurizing the reactor, may also be present.

As a first constituent of the biphasic liquid system an aqueous phase is present. In the aqueous phase the catalyst is preferably dissolved. The second constituent of the biphasic system is an organic phase which is at least partially immiscible with the aqueous phase. In the context of the present invention the term "at least partially immiscible" means that two distinct liquid phases are visible to the naked eye of an observer.

Under the proviso of at least partial immiscibility with the aqueous phase under the conditions (such as temperature, pressure, dissolved substances and molar fractions of aqueous and organic phases) under which the extractive step a) is performed the second, organic phase comprises a solvent selected from the group comprising esters, lactones, ethers and/or cyclic ethers. The solvent should be chosen to award a desired lignin extraction under the other boundary conditions of the method such as solvent recovery temperatures and the like. Examples for esters include bio-based polypropanediol di-esters with fatty acids and diethyl succinate. An example for a bio-based ether is an end-capped polypropylene glycol polyether. An example for a bio-based cyclic ether is methyltetrahydrofuran.

A further proviso is that said solvent does not comprise a hydroxyl group or hydroxyl groups. This is based on the reasoning that OH groups might lead to undesired side reactions with other alcohols, the lignocellulose or with components extracted therefrom during the method according to the invention.

Examples for the carboxylic acid catalyst include monocarboxylic acids, dicarboxylic acids and tricarboxylic acids such as formic acid, acetic acid, propanic acid, butyric acid, malonic acid, succinic acid, glutaric acid, adipinic acid, maleic acid, fumaric acid, lactic acid, malic acid, tartaric acid, citric acid and/or oxalic acid. An advantage of using carboxylic acid catalysts is that they may be recovered from aqueous solutions by crystallization.

Included within the term "carboxylic acid catalyst" are monoesters of carboxylic acids and diesters of carboxylic acids, provided that there is at least one remaining COOH functional group present in the molecule.

The present invention is further elucidated by way of the following preferred embodiments. They may be combined freely unless the context clearly indicates otherwise.

In one embodiment of the method according to the invention step a) is performed at a temperature of ≥ 85 °C to ≤ 150 °C. Below this temperature range the reaction rate might be slower than desired and above this range cellulose depolymerization might occur to an undesired extent. A preferred temperature range is ≥ 125 °C to ≤ 140 °C.

In another embodiment of the method according to the invention step a) is performed under a pressure of ≥ 5 bar to ≤ 25 bar. Preferred ranges are ≥ 10 bar to ≤ 20 bar. Pressurizing the reaction system can afford the biphasic liquid system even if the process temperature is above the boiling point of one of the constituents of the biphasic system. For example, when using 2-MTHF the pressure may be raised to a level at which 2-MTHF is liquid at 140 °C. The pressure may be applied via gases such as CO_2 or N_2.

In another embodiment of the method according to the invention step a) is performed for a duration of ≥ 2 hours to ≤ 6 hours. A preferred duration is ≥ 2.5 hours to ≤ 3.5 hours.

In another embodiment of the method according to the invention the carboxylic acid catalyst is selected from the group comprising dicarboxylic acids and/or monoesters of dicarboxylic acids. It is preferred that the carboxylic acid catalyst comprises oxalic acid and most preferred that that the carboxylic acid catalyst is oxalic acid.

In another embodiment of the method according to the invention the carboxylic acid catalyst is present in an amount of ≥ 0.5 weight-% to ≤ 2 weight-% in the first, aqueous phase. The weight percentage is to be understood as the amount of the catalyst in the aqueous phase before contacting the aqueous phase with other phases such as the organic phase or the lignocellulosic substrate. Preferably, the catalyst amount is ≥ 0.9 weight-% to ≤ 1.8 weight-%. Alternatively, the catalyst loading may be expressed as the molar concentration in the total liquid volume of the first, aqueous phase. For example, the catalyst loading may be ≥ 0.05 M (in total liquid volume) to ≤ 0.15 M (in total liquid volume).

In another embodiment of the method according to the invention the solvent in the second, organic phase comprises 2-methyltetrahydrofuran and/or 3-methyltetrahydrofuran. It is preferred that the solvent in the second, organic phase is 2-methyltetrahydrofuran and/or 3-methyltetrahydrofuran. Most preferred is 2-methyltetrahydrofuran (2-MTHF) as an extractive phase, as it can be derived from biomass, exhibits a miscibility gap with water, and has a boiling point (ca. 80 °C) that enables straightforward separation and recycling by distillation. A combination of 2-MTHF as the solvent in the second, organic phase and oxalic acid as the carboxylic acid catalyst has proven to be particularly suited for carrying out the method according to the invention.

In another embodiment of the method according to the invention the method further comprises the steps:

b1) at least partially removing the first, aqueous phase from the reaction vessel;

b2) reducing the volume and/or lowering the temperature of the first, aqueous phase obtained in step b1) to at least partially obtain the carboxylic acid catalyst in solid form; and

b3) re-entering the carboxylic acid catalyst obtained in step b2) into the first, aqueous phase of the reaction vessel in step a).

With the additional procedure of steps b1) to b3) a recycling of the carboxylic acid catalyst is effected. For example, the catalyst may be recovered by crystallization. The sugar-containing mother liquor can then be further processed.

In another embodiment of the method according to the invention the method further comprises the steps:

c1) at least partially removing the second, organic phase from the reaction vessel;

c2) at least partially removing the solvent from the second, organic phase obtained in step c1) to obtain a solvent free from dissolved substances; and

c3) re-entering the solvent obtained in step c2) into the second, organic phase of the reaction vessel in step a).

With the additional procedure of steps c1) to c3) a recycling of the organic solvent is possible. At the same time the lignin dissolved therein is collected. Examples for suitable solvent removal techniques include distillation and membrane separation.

The aforementioned steps of group b1) to b3) and of group c1) to c3) may be performed sequentially. It is also possible to perform them independent of each other. Another example for a suitable procedure is to perform a batch run according to step a), remove the obtained cellulose-pulp by filtration and then to recover and recycle the catalyst and the organic solvent.

The present invention will be further described with reference to the following examples and figure without wishing to be limited by them.

The overall process concept is shown schematically in FIG. 1 in which lignocellulosic biomass is subjected to mild ($T \leq 150$ °C) carboxylic acid catalyzed hydrolysis in a biphasic reaction mixture comprising an aqueous phase and an immiscible organic phase. More specifically, a reaction vessel 10 is charged with a biphasic reaction mixture comprising a first, aqueous phase 12 and an immiscible, second organic phase 11 in the form of a cyclic ether such as 2-methyltetrahydrofuran (2-MTHF). Furthermore, the aqueous phase 12 comprises a dicarboxylic acid such as oxalic acid as a catalyst. The temperature within the reaction vessel 10 may be preferably chosen to be between 80 °C and 140 °C.

The reaction vessel 10 is charged with a lignocellulosic biomass by way of stream 13. The mild conditions together with the choice of, for example, oxalic acid as catalyst lead to the selective hydrolysis of amorphous hemicellulose yielding soluble sugars (mostly xylose) in the aqueous phase 12. Likewise, lignin is dissolved in the organic phase 11. The product mixture is removed from the reaction vessel 10 via stream 14 and entered into a first separation unit 15.

The organic phase is entered as stream 16 into a second separation unit 19 which may be a distillation unit. Recovered organic phase is recycled into reaction vessel 10 via stream 21. Lignin obtained in the second separation unit 19 is carried out as stream 23.

The catalyst may be separated from the aqueous stream 18 leaving the first separation unit 15 by crystallization in a third separation unit 20 before further processing and is recycled into the process via stream 22. Hemicellulose sugars may be extracted from the aqueous stream in the third separation unit 20 as stream 24.

As the hemicellulose "glue" of the biomass composite material is removed by the depolymerization reaction occurring in the reaction vessel 10, the remaining biopolymers cellulose and lignin separate from each other owing to their very different structures and solubility properties. The cellulose stays insoluble as solid pulp and can be filtered off as stream 17 from the fist separation unit 15 for subsequent enzymatic hydrolysis into glucose. To this end, the pulp should be free of lignin, which is extracted in situ into the organic phase 11 of the biphasic reaction system as already described.

Examples

Materials: Oxalic acid, 2-methyltetrahydrofuran (2-MTHF), Avicel®, and reagents for PAHBAH colorimetric method, were obtained from Sigma-Aldrich, and were used without further purification. Beech wood with a defined size (0.5–0.8 mm) was used. Accellerase-1500® was supplied by Genencor (the Netherlands).

Standard procedure for lignocellulose fractionation: Wood loadings (50–100 gL^{-1}) were suspended in water with oxalic acid (0.1 M) (5 mL). As organic phase, 2-MTHF (5 mL) was added, and the mixture transferred to a stainless steel high pressure reactor and pressurized with CO_2 (10–20 bar). The temperature was set between 125–150 °C for the indicated reaction time. After cooling and depressurization of the reactor, the organic phase was separated by decantation and 2-MTHF was evaporated to obtain the lignin fraction. The aqueous phase was filtered to isolate the cellulose pulp, and xylose and glucose concentrations were determined in the aqueous phase. The solid residue (mostly pulp) was washed with ethanol and dried until constant weight.

Enzymatic procedure: Cellulose-pulp or Avicel® (20 gL^{-1}) were suspended in citrate buffer pH 4.5 (20 mL), at 50 °C for several hours. Accellerase-1500® was then added (1 v%). Aliquots of the reactions were taken, heated to 100 °C for 5 minutes to denaturalize the enzymes and stop the process, and stored at -20 °C until colorimetric analysis was conducted. The amount of reducing ends was determined using the PABAH method (M. Lever, *Anal. Biochem.* 1972, 47, 273–279) on basis of a calibration curve as previously described (vom Stein, T., Grande, P., Sibilla, F., Commandeur, U., Fischer, R., Leitner, W., Domínguez de María, P. *Green Chem.* 2010, 12, 1844–1849).

Xylose and glucose determination (HPLC): HPLC measurements were carried out on a Jasco HPLC equipped with a SUGARSH1011 column with 0.01 wt% aqueous acetic acid solution as eluent. Flow rate was set to 0.6 mL min^{-1} and samples of 30 μL were injected. Amounts of xylose and glucose present in the hemicellulose fraction were determined based on calibration curves built with commercially available real substrates.

Procedure for oxalic acid recycling: The aqueous phases from a representative experiment for lignocellulose fractionation were concentrated 4-fold by evaporating water. The concentrated aqueous solution was stored at 10 °C for 15 h. Under these conditions up to 85% of oxalic acid crystallized in the form of needle-like crystals. The crystals were filtered off and subjected to HPLC and NMR analysis, and subsequently re-used directly in the process.

In order to validate the process scheme as outlined above and in connection with FIG. 1, beech wood was taken as a prototypical lignocellulosic substrate with an approximate (variable) composition of 15–25% hemicellulose, 15–30% lignin, and 40–50% cellulose, together with some other minor components (salts, waxes, etc). A first set of experiments was conducted with various variations of the multi-component reactive system and at different temperatures (85–150 °C). The reactions were carried out either under reflux conditions or in high-pressure reactors in the presence of additional CO_2 pressure to ensure a liquid reactive system at temperatures above the solvent's boiling point. Representative results are listed in Table 1.

Table 1: Oxalic acid-catalyzed fractionation of beech wood in an aqueous/organic biphasic reaction system. Typical reaction conditions: beech wood (0.5–0.8 mm, 100 g L^{-1} in aqueous phase), oxalic acid 0.1 M (in total liquid volume), biphasic system water–2-methyltetrahydrofuran (2-MTHF) (1:1 v/v, 10 mL total), 6 hours reaction time.

Entry	Organic phase	T (°C)	p CO_2 (bar)[a]	Soluble Sugars[b] Xylose (wt%)	Soluble Sugars[b] Glucose (wt%)	Lignin (wt%)[c]	Solid residue (wt%)[d]
1	2-MTHF	85	Reflux	4.0	n.d.	4.0	84
2	---	125	Reflux	9.4	n.d.	4.0	70
3	2-MTHF	125	10	17.7	2.0	13.5	58.8
4	2-MTHF	125	20	15.4	2.2	16.0	50.0
6	2-MTHF	140	10	16.3	3.2	12.1	57.8
7	2-MTHF	145	10	18.0	5.8	13.5	50.9
8	2-MTHF	150	10	15.0	8.5	11.4	50.2

a) CO_2 pressure was set at room temperature. b) Xylose and glucose (wt%) were analyzed by HPLC c) Lignin obtained after removal of 2-MTHF under reduced pressure. d) Solid residue (mostly pulp in entries 3-8) was obtained by filtrating the aqueous phase, washed with ethanol, and dried until constant weight.

The depolymerization of hemicellulose was monitored via the formation of soluble sugars and in particular xylose in the aqueous phase. At temperatures below 100 °C no significant amounts of xylose were detected (table 1, entry 1). Under monophasic conditions in water only, the depolymerization at 125 °C led to outcomes in the range of those reported in US 3,586,537 for analogous aqueous processes (table 1, entry 2). Significantly higher yields of xylose were obtained under biphasic conditions in combination with 2-MTHF as second organic phase (table 1, entries 3-8). The maximum yields of xylose were fully consistent with the reported hemicellulose composition in beech wood (A. Wisogel, S. Tyson, D. Johnson, *Handbook on bioethanol: Product and Utilization* (Ed. C.E. Wyman), Taylor & Francis, Washington, 1996) indicating that the depolymerization was practically complete under these conditions. ^{1}H- and ^{13}C-NMR-spectroscopic analysis revealed that the aqueous solution contained monomeric sugars in high purity, consisting mainly of xylose together with small amounts of glucose.

A second set of experiments was conducted in order to compare the yields of xylose in the biphasic system water/2-MTHF under 10 bar of CO_2 at different temperatures and at two different reaction times of 3 hours and 6 hours. It was observed that at the shorter reaction time the yield of xylose increased significantly by increasing the temperature from 125 °C to 140 °C. At 6 hours reaction time, the yield decreases again at 150 °C, presumably due to secondary transformation/decomposition of the primary sugar product. In both cases, the optimum yields approach the theoretical limit defined by the hemicellulose content of beech wood material. Results are listed in Table 2.

Table 2: Xylose yields at different temperatures for two different reaction times.

Temperature (°C)	Xylose (wt%) (3 h)	Xylose (wt%) (6 h)
125	11	16
140	17	16
150	18	14

In addition to the xylose, glucose was also formed during the hydrolysis. The amount of glucose was in the range of 2–3% at temperatures between 125–140 °C. This level is consistent with the glucose fraction in hemicellulose. However, glucose formation increased rapidly when temperatures higher than 140 °C were set in the reactor. This additional glucose production is likely to have arisen from cellulose depolymerization, which is known to start at temperatures of 150 °C in the presence of organic acids. Thus, higher temperatures lead to a decrease in selectivity for biomass fractionation, which adversely affects the potential as feedstock for the three main (pure) components of lignocellulose. Conclusively, treatment at 140 °C for shorter times up to 3 hours defines the optimal compromise for a selective fractionation of beech wood in the present system without significant pulp degradation. Results are listed in Table 3.

Table 3: Glucose yields at different temperatures for two different reaction times.

Temperature (°C)	Glucose (wt%) (3 h)	Glucose (wt%) (6 h)
125	1	2
140	2.5	3
150	6	8

In addition to the hemicellulose sugars, the aqueous phase in the experiments contained large amounts of the oxalic acid catalyst. Gratifyingly, this can be recovered and re-used in an efficient and clean manner. Under non-optimized conditions, it was observed that by reducing the volume of the aqueous phase to one fourth and cooling it down to 4 °C, approximately 85% of the originally charged oxalic acid crystallized within 15 hours and could be recovered by simple filtration. The oxalic acid showed high purity and could be used as catalyst again without further processing or activation.

Efficient extraction of lignin into the 2-MTHF phase was achieved particularly when additional CO_2 pressure was applied in the reactor to ensure the presence of a liquid organic phase at the high temperature (Table 1, entries 3–8). The beneficial effect of pressure is consistent with findings from other pre-treatment methods such as the organosolv process (R.P. Chandra, R. Bura, W.E. Mabee, A. Berlin, X. Pan, J.N. Saddler, *Adv. Biochem. Engin/Biotechnol.* 2007, 108, 67–93; Y.H. Zhang, M. E. Himmel, J.R. Mielenz, *Biotechnol. Adv.* 2006, 24, 452–481; 6 N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple, M. Ladisch, *Biores. Technol.* 2005, 96, 673–686; Y. Sun, J. Cheng, *Biores. Technol.* 2002, 83, 1–11). The amount of lignin recovered directly after evaporation of 2-MTHF reached up to 60–70% of the theoretical value expected from average beech wood composition. The optical appearance and the ^{1}H-NMR spectra of this lignin were compared to commercially available organosolv material. The colour of the material as well as the much better resolved NMR signals obtained suggest that the mild processing conditions of the present method lead to less degradation of lignin and lignin derivatives. The 2-MTHF removed the lignin from the acidic aqueous phase efficiently and can be distilled and re-used in the process.

The solid residue (mostly pulp) constituted the third feedstock stream from the separation method which was readily isolated by filtration from the reaction mixture after removal of the 2-MTHF, and washed with ethanol. The mass of the crude material (50-58 wt%, Table 1) corresponds fairly well with the amount of cellulose in beech wood.

In the following table 4 the rate of formation of reducing-end sugars, which is indicative of cellulose depolymerization, is compared for various cellulose materials using the commercially available enzymatic preparation Accellerase-1500® (Genencor) which is composed of different glycosidases. As expected, beech wood is practically not hydrolyzed by Accellerase-1500® at all, reinforcing the importance of pre-treatment to liberate the carbohydrate polymers. Similarly, the pulp obtained under monophasic conditions (Table 1, entry 2) was also not hydrolyzed, presumably reflecting the known inhibitory effect of the co-precipitated lignin on the enzyme activity. In sharp contrast, the pulp isolated from the biphasic water/2-MTHF system as described above for the entry 3 in Table 1 was depolymerized readily with productivities of about 3.5 g of soluble reducing-end sugars per litre and hour. The rate is comparable to that observed for the commercial high purity microcrystalline cellulose Avicel®.

Table 4: comparison of cellulose depolymerization

Reaction time (h)	Soluble reducing-end sugars per litre and hour			
	Avicel (comparison example)	Pulp (biphasic; according to invention)	Pulp (monophasic; comparison example)	Beech wood (comparison example)
0	0	0	0	0
1	5	3.8	0.1	0
2	6	4	0.9	0
3	7.5	6	0.8	0.2

Claims

1. A method for processing lignocellulose, comprising the step of:

a) contacting a lignocellulosic substrate with a biphasic liquid system in a reaction vessel,

the biphasic liquid system comprising a first, aqueous phase and a second, organic phase which is at least partially immiscible with the first, aqueous phase under the conditions under which step a) is performed,

wherein the first, aqueous phase further comprises a carboxylic acid catalyst and

wherein the second, organic phase comprises a solvent selected from the group comprising esters, lactones, ethers and/or cyclic ethers, with the proviso that said solvent does not comprise a hydroxyl group or hydroxyl groups.

2. The method according to claim 1, wherein step a) is performed at a temperature of ≥ 85 °C to ≤ 150 °C.

3. The method according to claim 1 or 2, wherein step a) is performed under a pressure of ≥ 5 bar to ≤ 25 bar.

4. The method according to one of claims 1 to 3, wherein step a) is performed for a duration of ≥ 2 hours to ≤ 6 hours.

5. The method according to one of claims 1 to 4, wherein the carboxylic acid catalyst is selected from the group comprising dicarboxylic acids and/or monoesters of dicarboxylic acids.

6. The method according to claim 5, wherein the carboxylic acid catalyst comprises oxalic acid.

7. The method according to one of claims 1 to 6, wherein the carboxylic acid catalyst is present in an amount of ≥ 0.5 weight-% to ≤ 2 weight-% in the first, aqueous phase.

8. The method according to one of claims 1 to 7, wherein the solvent in the second, organic phase comprises 2-methyltetrahydrofuran and/or 3-methyltetrahydrofuran.

9. The method according to one of claims 1 to 8, further comprising the steps:

b1) at least partially removing the first, aqueous phase from the reaction vessel;

b2) reducing the volume and/or lowering the temperature of the first, aqueous phase obtained in step b1) to at least partially obtain the carboxylic acid catalyst in solid form; and b3) re-entering the carboxylic acid catalyst obtained in step b2) into the first, aqueous phase of the reaction vessel in step a).

10. The method according to one of claims 1 to 9, further comprising the steps:

c1) at least partially removing the second, organic phase from the reaction vessel;

c2) at least partially removing the solvent from the second, organic phase obtained in step c1) to obtain a solvent free from dissolved substances; and

c3) re-entering the solvent obtained in step c2) into the second, organic phase of the reaction vessel in step a).

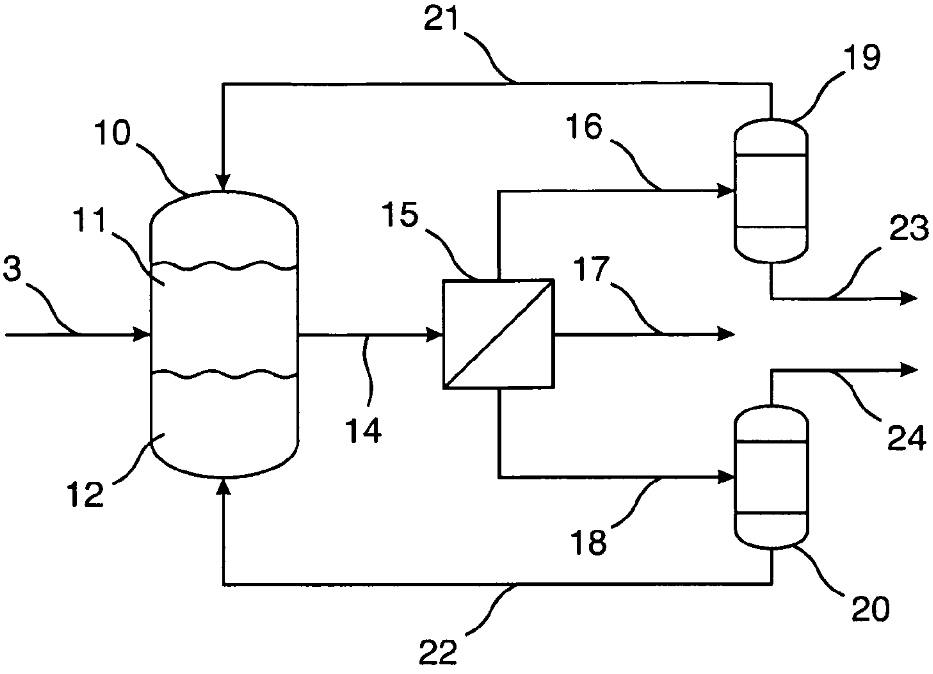

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/000656

A. CLASSIFICATION OF SUBJECT MATTER
INV. D21C3/20
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
D21C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 211 558 A2 (BIODYNE CHEMICALS INC [US]) 25 February 1987 (1987-02-25) claims 1-10; figure 1; examples 1-4 -----	1
A	US 4 409 032 A (PASZNER LASZLO [CA] ET AL) 11 October 1983 (1983-10-11) example 4 -----	1-10
A	EP 0 043 342 A1 (BATTELLE MEMORIAL INSTITUTE [CH]) 6 January 1982 (1982-01-06) claims 1-14; examples 1-9 -----	1-10
A	US 2001/018956 A1 (STOHRER JURGEN [DE] ET AL) 6 September 2001 (2001-09-06) the whole document ----- -/--	1-10

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search 28 March 2012	Date of mailing of the international search report 04/05/2012
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Karlsson, Lennart

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/000656

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 730 837 A (BLACK STUART K [US] ET AL) 24 March 1998 (1998-03-24) cited in the application the whole document -----	1-10
A	US 4 594 130 A (CHANG PEI-CHING [CA] ET AL) 10 June 1986 (1986-06-10) the whole document -----	1-10
A	US 4 087 318 A (SAMUELSON HANS OLOF ET AL) 2 May 1978 (1978-05-02) the whole document -----	1-10
A	WO 2007/008689 A2 (BIOPULPING INT INC [US]; AKHTAR MASOOD [US]; KENEALY WILLIAM ROBERT [U) 18 January 2007 (2007-01-18) cited in the application the whole document -----	1-10
A	WO 2010/046051 A2 (ENI SPA [IT]; BIANCHI DANIELE [IT]; FRANZOCI GIULIANA [IT]; ROMANO ANN) 29 April 2010 (2010-04-29) the whole document -----	1-10

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/000656

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0211558 A2	25-02-1987	AU 6049386 A	19-03-1987
		BR 8603731 A	10-03-1987
		CN 86105219 A	25-02-1987
		DK 353886 A	20-03-1987
		EP 0211558 A2	25-02-1987
		FI 863069 A	27-01-1987
		JP 62069893 A	31-03-1987
		NO 863018 A	27-01-1987
US 4409032 A	11-10-1983	BE 870112 A2	18-12-1978
		CA 1100266 A1	05-05-1981
		CS 227660 B2	14-05-1984
		HU 177362 B	28-09-1981
		IN 150626 A1	20-11-1982
		SU 1194282 A3	23-11-1985
		US 4409032 A	11-10-1983
		ZA 7804979 A	28-11-1979
EP 0043342 A1	06-01-1982	BR 8103885 A	09-03-1982
		CA 1167204 A1	15-05-1984
		DE 3161585 D1	12-01-1984
		EP 0043342 A1	06-01-1982
		FI 811894 A	21-12-1981
		JP 57051889 A	26-03-1982
		OA 6842 A	28-02-1983
		US 4511433 A	16-04-1985
		ZA 8104137 A	24-11-1982
		ZW 14381 A1	24-03-1982
US 2001018956 A1	06-09-2001	NONE	
US 5730837 A	24-03-1998	NONE	
US 4594130 A	10-06-1986	NONE	
US 4087318 A	02-05-1978	NONE	
WO 2007008689 A2	18-01-2007	AU 2006269326 A1	18-01-2007
		CA 2614704 A1	18-01-2007
		EP 1937892 A2	02-07-2008
		JP 2009500537 A	08-01-2009
		US 2009194243 A1	06-08-2009
		WO 2007008689 A2	18-01-2007
WO 2010046051 A2	29-04-2010	EA 201100551 A1	30-12-2011
		EP 2350300 A2	03-08-2011
		WO 2010046051 A2	29-04-2010