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(54) Title: SYSTEM AND METHOD FOR SEPARATING CARBOHYDRATE AND LIGNIN IN HYDROLYSATE OF BIOMASS

(57) Abstract: A system and method are disclosed for pretreating biomass and for processing the hydrolysate of biomass. In order to improve the efficiency of a biorefinery, carbohydrate and lignin in the hydrolysate of biomass are separated which may help improve the efficiency of the biorefinery process and also provide more efficient use of the carbohydrate and lignin.



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SYSTEM AND METHOD FOR SEPARATING CARBOHYDRATE AND LIGNIN IN HYDROLYSATE OF BIOMASS

RELATED APPLICATIONS

[0001] This application claims priority of U. S. Provisional Application No. 61/570,645 filed on December 14, 2011, the content of which is hereby incorporated into this application by reference.

I. Field of the Invention

[0002] This disclosure pertains to treatment of biomass to enhance conversion efficiency from biomass to biofuels, or other useful chemicals. More specifically, the disclosure relates to a method for improving conversion efficiency by separating carbohydrate and lignin in the hydrolysate of biomass.

II. Background

[0003] Cellulosic biomass is useful for generating biofuels such as ethanol and other valuable chemicals. Many cellulosic biomass such materials specifically known as lignocellulosic materials, or biomass, (e.g. wood and solid wastes), have been used as source material to generate carbohydrates, which in turn may be used to produce ethanol, as well as other products. However, large-scale utilization of plant biomass is hindered, at least in part, by the lack of technologies capable of efficiently converting the biomass into component fractions or reactive intermediates at a low cost. For example, most plant biomass is resistant to the digestion by cellulase, which may lead to low cellulose hydrolysis yields.

[0004] Pretreatment of biomass may render the biomass more amenable to enzymatic digestion. For instance, biomass components (e.g., lignin) that impede access to cellulase enzymes may be removed during pretreatment. Pretreatment may also cause structural changes (e.g. particle size, porosity, surface area) to the biomass which may render them more accessible to enzymes. Various biomass pretreatment technologies have been developed. Examples of these developments include use of dilute acids or bases, steam explosion, autohydrolysis, controlled pH, AFEX, and aqueous ammonia pretreatment.

[0005] Autohydrolysis pretreatment employs hot water or steam to pretreat biomass. However, high pretreatment severity (e.g. temperature >190°C) is generally

required to produce digestible substrate which may result in high losses of hemicellulose sugars. For instance, depending on residence time, xylose losses can be as high as 25%, or even higher. In addition, inhibitors released from the biomass and produced in the course of sugar and lignin degradation may negatively affect the qualities of the insoluble materials, such as substrate fermentability and digestibility.

[0006] In conventional steam pretreatment in which the residence time of the solids and liquid is the same, whether operated in batch or continuous mode, dissolved biomass components may degrade once they are dissolved or suspended in solution. In addition, solubilized lignin and hemicellulose components may precipitate during cooling, which decreases the reactivity of the biomass to enzymatic hydrolysis.

[0007] One approach for improving pretreatment effectiveness involves washing of the solid biomass after closed-system pretreatment ("post-washing"). Post-washing at high temperatures, for example, at 140°C, helps produce reactive biomass material and also removes some lignin and hemicellulose solubilization products. The amount of lignin and hemicellulose solubilization products removed in post-washing may not be as much as the amount that would be removed if washing were done at pretreatment reaction temperatures. Furthermore, once-through washing typically dilutes solubilized components, making them more expensive to recover or process in subsequent steps. Although post-washing of solid biomass at moderate temperatures (e.g., 100°C) and under atmospheric pressure may help eliminate certain complexities, it is not very efficient in producing adequate yield of the biomass solids and makes achieving sterilization more difficult.

[0008] Another approach for enhancing pretreatment effectiveness involves flowing hot water, or acid, through the solid biomass, also known as flowthrough pretreatment. Flowthrough pretreatment with hot water, or very dilute acid, may effectively remove hemicellulose and lignin, and may generate highly active substrate (Liu & Wyman, 2003, 2004). For example, hot water flowthrough pretreatment removes significant amount of dissolved hemicellulose and lignin thus avoiding precipitation. When flowthrough pretreatment is carried out with hot liquid at a temperature of, for example, between 120°C and 240°C, the reactivity of the resulting biomass solids are several-fold greater than that of a closed-system control. However, conventional flowthrough operation uses too much energy and water. Moreover, the presence of lignin in the carbohydrate mixture decreases the efficiency of the process.

SUMMARY

[0009] The disclosed instrumentalities advance the art by providing systems and methods for separating carbohydrate and lignin in the hydrolysate of biomass. The disclosed methods may improve the economics of biorefinery by enhancing the conversion efficiency from biomass to biofuels or other chemicals. More particularly, the disclosed methods may provide for more efficient use of carbohydrate and lignin in the flowthrough pretreatment hydrolysate of biomass. In one aspect, the disclosed systems and methods may be employed to increase the efficiency of lignocellulosic biomass utilization. In another aspect, more lignin may be recovered from the biomass. In another aspect, the lignin recovered according to the disclosed methods is likely to be more pure and of higher quality. Moreover, because substantial amount of lignin is removed prior to fermentation, inhibition of fermentation by lignin may be significantly reduced, which, in turn, may enhance the efficiency and yield of biofuel production from biomass.

[0010] In one embodiment, a system for treating biomass may contain a vessel (also known as a mixer or a reactor) for holding a hydrolysate of a biomass. An agent such as a hemicellulase enzyme, a mixture of enzymes, or an acid may be added to the hydrolysate in the vessel, where reaction between the agent and said hydrolysate may cause formation of a first solid phase and a first liquid phase.

[0011] In one aspect, the first solid phase may contain greater than 50%, 60%, 70%, 80%, or even 90% of the total lignin in the hydrolysate. In another aspect, less than 15%, 20%, or 30% of the total fermentable sugars in the hydrolysate are precipitated and are present in the solid phase. The vessel may have a means for regulating the temperature such that the reaction temperature inside the vessel may be controlled. For instance, the reactants may be heated or cooled to separate the first solid phase and the first liquid phase more efficiently.

[0012] The system may contain a first means for separating the first solid phase from the first liquid phase. The first separation means may be an integral part of the vessel, or alternatively, the first separation means may be a standalone component of the system.

[0013] After being separated from the first solid phase, the first liquid phase may be transferred to a fermenter where the contents of the first liquid phase may be subject to fermentation to produce various fermentation products such as, but not limited

to ethanol. In another embodiment, after being separated from the first solid phase, the first liquid phase may be subject to another separation process wherein a second separating means separates xylooligomers having low degree of polymerization (low DP xylooligomers) from xylooligomers having high degree of polymerization (high DP xylooligomers). The high DP xylooligomers may be transferred via a first conduit back to the vessel wherein the residual lignin in the high DP xylooligomers may be separated. Alternatively, the high DP xylooligomers may be transferred to the fermenter to produce biofuels and/or other chemicals. Examples of low DP xylooligomers may include, for example those xylooligomers containing 2-5 units of xylose. By way of example, low DP xylooligomers may be used as food ingredients, or as ingredients of prebiotics, among others. Prebiotics may help stimulate the growth and/or activity of beneficial bacteria in the digestive system of human or animals.

[0014] The solid phase that comes out from the first separation means may be subject to hydrolysis by a dilute acid which generates a second liquid phase and a second solid phase. The second liquid phase may contain carbohydrates that are precipitated along with lignin in the first solid phase. The recovered carbohydrates may be transferred to the fermenter via a second conduit. The second solid phase may contain lignin. In one aspect, the percentage of lignin in the second solid phase is higher than the percentage of lignin in the first solid phase.

[0015] In another embodiment, a system for treating biomass may contain a vessel (or a bioreactor) for holding a hydrolysate of a biomass. An agent such as a microorganism capable of fermenting carbohydrate may be mixed with the hydrolysate in the vessel. Examples of the microorganism may include but are not limited to, for example, *Thermoanaerobacterium saccharolyticum*. The microorganism may convert the carbohydrate into ethanol and/or other chemicals. As the fermentation reaction progresses, a third liquid and a third solid phase may form. The third liquid phase may contain ethanol and/or other chemicals, while the third solid phase may contain lignin and/or other insoluble materials.

[0016] The system may also contain a separation means for separating the third solid phase from the third liquid phase formed in the bioreactor. This separation means may be part of the bioreactor or it may be a standalone component of the system.

[0017] After being separated from the third liquid phase, the third solid phase may be subject to hydrolysis by a dilute acid, which may, in turn, create a fourth liquid

phase and a fourth solid phase. The fourth solid phase may contain lignin. The fourth liquid phase may contain carbohydrates and may be transferred via a third conduit back to the bioreactor where the carbohydrates are fermented.

[0018] In another aspect, the recovered lignin may be used to produce carbon fiber. Lignin may also be used as a filler/component for plastics production or a raw material for chemical production. The solid phase may be processed to further fractionate the lignin components.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 is a flow chart showing one embodiment of the disclosed system.

[0020] Figure 2 is a flow chart showing another embodiment of the disclosed system.

[0021] Figure 3 is a flow chart showing another embodiment of the disclosed system.

[0022] Figure 4 shows differential precipitation of carbohydrate and lignin under various acid concentrations.

DETAILED DESCRIPTION

[0023] The present disclosure provides systems and methods for separating carbohydrate and lignin from a hydrolysate of biomass. The methods may be applied to any biomass that contains carbohydrate and lignin or processed products derived from these biomass, for example, hydrolysates of biomass.

[0024] In one embodiment, as shown in Fig. 1, hydrolysate of biomass (stream 1) and an acid or a hemicellulase enzyme (stream 2) may be added into a mixer or a reactor, where the hydrolysate and the acid and/or enzyme are mixed. Example of the acid may include but are not limited to hydrochloric acid and sulfuric acid. The mixture (stream 3) may be subject to phase separation in which liquids and solids are separated. In one aspect, the phase separation may be carried out in a separate container other than the mixer or reactor. In another aspect, the phase separation may take place in the same mixer (or reactor).

[0025] After the phase separation, the liquid portion may be sent to a fermentation vessel for fermentation (stream 6) or it may be further separated (stream 4) into low DP (DP stands for "degree of polymerization") xylooligomers (stream 7) and

high DP xylooligomers (stream 8 or stream 11). In one aspect, the high DP xylooligomers may be recycled to the mixer/reactor (stream 8). In another aspect, the high DP xylooligomers may be directly sent to fermentation (stream 11). The solid portion (stream 5) may be subject to hydrolysis by a dilute acid. The hydrolysis product may be separated into a liquid phase (stream 10) and a solid phase (stream 9). The liquid phase (stream 10) may contain mostly carbohydrate and the solid phase (stream 9) may contain mostly lignin. The carbohydrate stream (stream 10) may be sent to a fermentation vessel where the carbohydrate is converted to ethanol or other fermentation products (stream 12). In one aspect, the lignin stream may be washed to remove trace amount of carbohydrate that remains in the lignin stream.

[0026] In another embodiment, as shown in Fig. 2, hydrolysate (stream 1) and acid (stream 2) may be added into a container. The container may have an element to control the temperature. In one embodiment, the temperature inside the container may be maintained at 110 °C. The mixture is allowed to incubate in the container for a period of time such that hydrolysis of the biomass hydrolysate by the dilute acid can take place in the container. After the hydrolysis, the mixture (stream 3) may be subject to a separation process in which the liquid and solid phases are separated. The separation process may take place in a different container or in the same container where dilute acid hydrolysis takes place. After the separation step, the liquid stream (stream 5) may contain mostly carbohydrate and may be sent to a fermentation vessel where the carbohydrate is converted to ethanol or other fermentation products (stream 6). The solids stream (stream 4) contains lignin and may be used to recover lignin. The solids stream may be washed to remove trace amount of carbohydrate.

[0027] In another embodiment, as shown in Fig. 3, a hydrolysate (stream 1) and a medium (stream 2) are added into a bioreactor. The bioreactor is inoculated with one or more strains capable of hydrolyzing the carbohydrate and fermenting the sugars. The one or more strains may be, for example, *Thermoanaerobacterium thermosaccharolyticum* or *Thermoanaerobacterium saccharolyticum*. The bioreactor may be a batch reactor or a continuous reactor. The reacted mixture (stream 3) may be subject to a separation step to separate the liquid and solid phases. The separation step may be carried out in a separate vessel or may be incorporated into the bioreactor. After the separation step, the liquid stream (stream 4) may be subject to a process in which fermentation products are recovered. The solids portion (stream 5) may undergo dilute

acid hydrolysis. After the dilute acid hydrolysis, carbohydrate remains in the liquid phase (stream 6) while lignin remains in the solid stream (stream 7). In one aspect, the carbohydrate stream may be recycled back to the bioreactor. In another aspect, the lignin stream may be washed to remove trace amount of carbohydrate that remains in the lignin stream.

[0028] As used herein, "flowthrough" refers to a process wherein a liquid is added to or mixed with a solid or a semi-solid material and is incubated with the material for a period of time before leaving the solid or semi-solid material. During the course of the flowthrough, the liquid may solubilize, extract or otherwise bring along certain components of the biomass. Flowthrough pretreatment is distinguished from all other pretreatment configurations because the liquid phase could have a shorter residence time in the reactor than does the solid phase. Methods for reducing energy consumption and for effectively extracting usable sugar substrate from biomass are disclosed.

[0029] The term "biomass" generally refers to non-fossilized renewable materials that are derived from or produced by living organisms. For purpose of this disclosure, biomass may include animal biomass, plant biomass, and human waste and recycled materials, among others. Examples of animal biomass may include animal by-product and animal waste, etc. Plant biomass may be any plant-derived matter (woody or non-woody) that is available on a sustainable basis. Plant biomass may include, but is not limited to, agricultural crop wastes and residues such as corn stover, wheat straw, rice straw, sugar cane bagasse and the like, grass crops, such as switch grass and the like. Plant biomass may further include, but is not limited to, woody energy crops, wood wastes and residues such as trees, softwood forest thinnings, barky wastes, sawdust, paper and pulp industry residues or waste streams, wood fiber, and the like. In urban areas, plant biomass may include yard waste, such as grass clippings, leaves, tree clippings, brush, etc., vegetable processing waste, as well as recycled cardboard and paper products.

[0030] The terms "vessel" refers to a container that holds the biomass and one or more other reactants, wash, or enzymes, among others.

EXAMPLES

[0031] The following examples are provided for purpose of illustrating the instant disclosure and are not limiting.

EXAMPLE 1 DIFFERENTIAL PRECIPITATION OF LIGNIN AND CARBOHYDRATE IN PRETREATED HYDROLYSATE

[0032] In order to determine the appropriate concentration of the acid for precipitation of the various components, bagasse hydrolysate produced in a flowthrough pretreatment was concentrated by rotary evaporation. Different amounts of concentrated sulfuric acid were added to the hydrolysate to achieve different acid concentrations. The mixtures were incubated for about 30 minutes at room temperature. Precipitates were separated from the rest of the hydrolysate by centrifugation. The amounts of lignin and carbohydrate carbon in the precipitates as a percentage of total lignin and total carbohydrate carbon were analyzed. As shown in Fig. 4, more lignin was precipitated than carbohydrate under the acid concentrations tested.

EXAMPLE 2 DIFFERENTIAL PRECIPITATION OF LIGNIN AND CARBOHYDRATE AFTER HEMICELLULASE HYDROLYSIS

[0033] Bagasse hydrolysate produced in flowthrough pretreatment was concentrated by rotary precipitation. A few mills were taken and centrifuged. Supernatant was recovered after the centrifugation. 0.1 ml of Multifect xylanase was added to 3 ml of the supernatant along with 3 μ l of concentrated kanamycin solution. The mixture was incubated at 37 °C for 20 hours to allow precipitates to form. Xylan concentration and lignin carbon in the supernatant and precipitates were analyzed. As shown in Table 1, more than 80% lignin was precipitated. The precipitates contained a small amount of xylan.

TABLE 1: DIFFERENTIAL PRECIPITATION OF LIGNIN AND CARBOHYDRATE AFTER HEMICELLULASE HYDROLYSIS

	Xylan (g/L)	Lignin carbon (g/L)
Supernatant	35.77	0.82
Precipitate	1.5	3.59
Total	37.26	4.41
% Precipitated	4.02	81.4

CLAIMS

We claim:

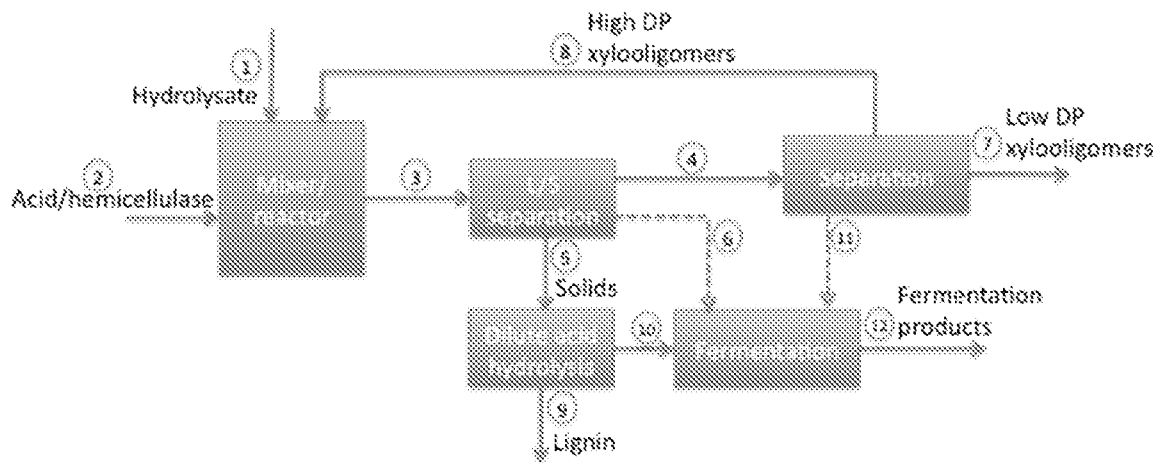
1. A system for treatment of biomass, said system comprising:
 - (a) a vessel for mixing an agent with a hydrolysate of said biomass, wherein reaction between said agent and said hydrolysate causes formation of a first solid phase and a first liquid phase in said vessel, said first solid phase comprising greater than 50% of total lignin and less than 30% of total fermentable sugar in said hydrolysate,
and
 - (b) a first means for separating said first solid phase from said first liquid phase.
2. The system of claim 1, wherein said agent is a member selected from the group consisting of an acid, a hemicellulase enzyme, and combination thereof.
3. The system of any one of the preceding claims, further comprising a means for controlling the temperature of the vessel.
4. The system of any one of the preceding claims, further comprising a fermenter, said first liquid phase being transferred to said fermenter.
5. The system of any one of the preceding claims, further comprising a second means for separating in said first liquid phase xylooligomers having low degree of polymerization (low DP xylooligomers) from xylooligomers having high degree of polymerization (high DP xylooligomers).
6. The system of any one of the preceding claims, further comprising a first conduit for transferring said high DP xylooligomers to said vessel.
7. The system of any one of the preceding claims, wherein said high DP xylooligomers are transferred to said fermenter.
8. The system of any one of the preceding claims, wherein said first solid phase is further subject to hydrolysis by a dilute acid, said hydrolysis generating a second liquid phase and a second solid phase, said second solid phase comprising lignin.

9. The system of any one of the preceding claims, further comprising a second conduit for transferring said second liquid phase to said fermenter.
10. A system for treatment of biomass, said system comprising:
- (a) a vessel for mixing an agent with a hydrolysate of said biomass, wherein reaction between said agent and said hydrolysate causes formation of a first solid phase and a first liquid phase in said vessel, said first solid phase comprising lignin, said liquid phase comprising ethanol, and said agent being a microorganism capable of fermenting carbohydrate to produce ethanol,
- and
- (b) a first means for separating said first solid phase from said first liquid phase.
11. The system of claim 10, wherein said first solid phase is subject to hydrolysis by a dilute acid, said hydrolysis generating a second liquid phase and a second solid phase, said second solid phase comprises lignin.
12. The system of any one of the preceding claims 10-11, further comprising a conduit for transferring said second liquid phase to said vessel.
13. A method for pretreatment of biomass, said method comprising:
- (a) mixing in a vessel a hydrolysate of said biomass with an agent, wherein reaction between said agent and said hydrolysate causes formation of a first solid phase and a first liquid phase in said vessel, said first solid phase comprising greater than 50% of total lignin and less than 30% of total fermentable sugar in said hydrolysate.
14. The method of claim 13, wherein said agent is a member selected from the group consisting of an acid, a hemicellulase enzyme, and combination thereof.
15. The method of any one of the preceding claims, further comprising a step of separating said first solid phase from said first liquid phase.
16. The method of any one of the preceding claims, further comprising the step of transferring said first liquid phase to a fermenter, wherein carbohydrate in said first liquid phase is fermented to produce ethanol in said fermenter.

17. The method of any one of the preceding claims, further comprising the step of separating in said first liquid phase xylooligomers having low degree of polymerization (low DP xylooligomers) from xylooligomers having high degree of polymerization (high DP xylooligomers).
18. The method of any one of the preceding claims, further comprising a step of transferring said high DP xylooligomers to said vessel.
19. The method of any one of the preceding claims, further comprising a step of transferring said high DP xylooligomers to said fermenter.
20. The method of any one of the preceding claims, further comprising a step of hydrolyzing said first solid phase using a dilute acid, said hydrolysis generating a second liquid phase and a second solid phase, said second solid phase comprising lignin.
21. The method of any one of the preceding claims, further comprising a step of transferring said second liquid phase to said fermenter.
22. A method for pretreatment of biomass, said method comprising:
- (a) mixing in a vessel a hydrolysate of said biomass with an agent, wherein reaction between said agent and said hydrolysate causes formation of a first solid phase and a first liquid phase in said vessel, said first solid phase comprising lignin, said liquid phase comprising ethanol, and said agent being a microorganism capable of fermenting carbohydrate to produce ethanol, and
 - (b) separating said first solid phase and said first liquid phase,
 - (c) hydrolyzing said first solid phase using a dilute acid solution, said hydrolysis generating a second liquid phase and a second solid phase, said second solid phase comprising lignin, and
 - (d) transferring said second liquid phase to said vessel for fermentation.

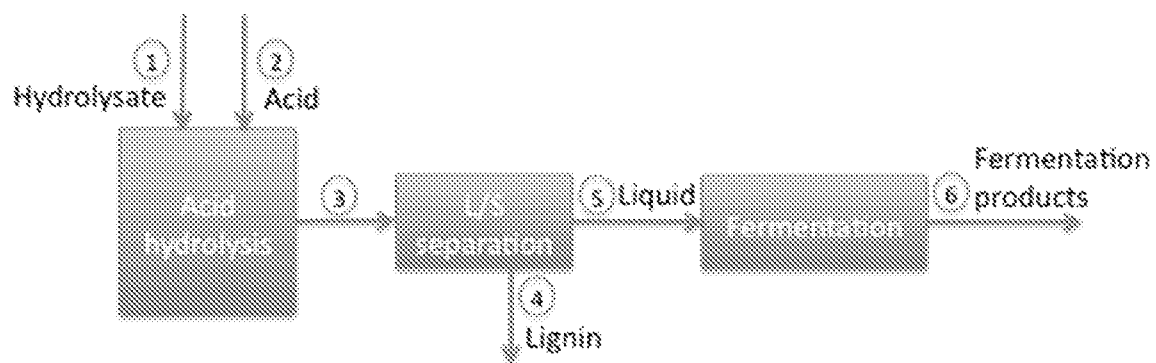
1/4

FIG. 1



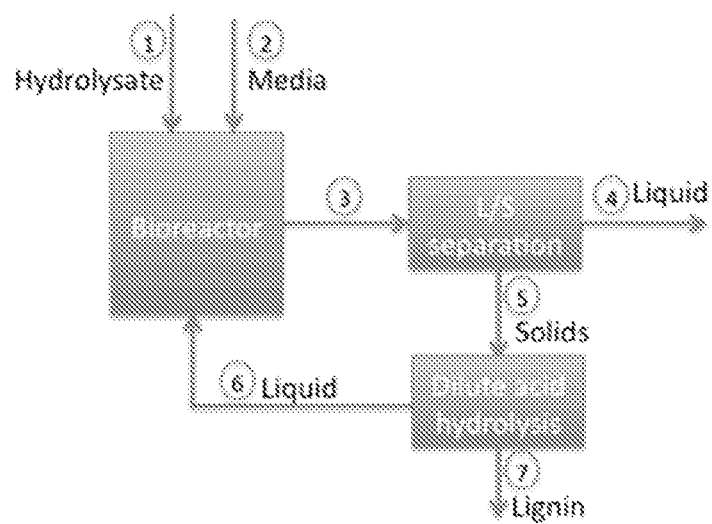
2/4

FIG. 2



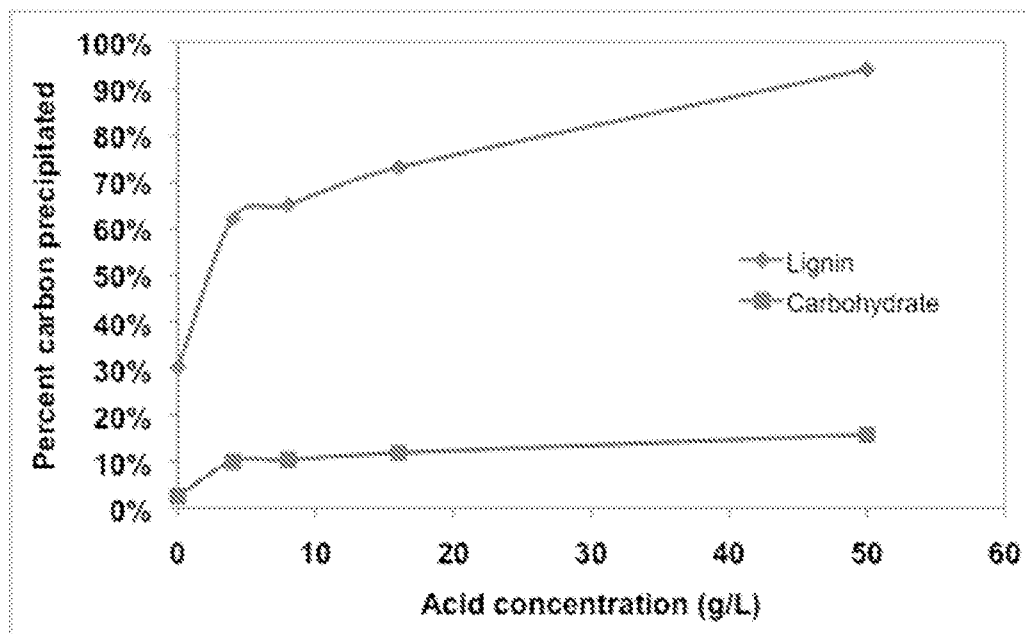
3/4

FIG. 3



4/4

FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/069854**A. CLASSIFICATION OF SUBJECT MATTER****C07C 7/148(2006.01)i, C07C 29/88(2006.01)1, C07C 29/94(2006.01)1, C07C 31/08(2006.01)1**

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)

C07C 7/148; C02F 3/00; C12P 7/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility modelsElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal), Google Scholar & Keywords: biomass, fermentable sugar, lignin, hydrolysate, acid, microorganism**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,612,286 A (SHERMAN, MICHAEL I. et al.) 16 September 1986 See abstract, column 1 line 25-40, column 2 lines 35-43, and claims 1-5.	1-3, 10-14, 22
X	LU, X. B. et al., Enzymatic Hydrolysis of Corn Stover after Pretreatment with Dilute Sulfuric Acid. Chem. Eng. Technol., 2007, Vol.30, No.7, pp. 938-944. See abstract, figure 2, 3.4.Effect of hemicel lulose, 4.3.6.Acid hydrolysis pretreatment, and 4.4. Biological pretreatment.	10-12, 22
A		1-3, 13-14
A	CARA, CRISTOBAL et al., Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification. Bioresource Technology, 2008, Vol.99, pp.1869-1876. See abstract, Table 1, 3.2. Dilute acid pretreatment, and 4.Conclusion.	1-3, 10-14, 22
A	CARA, CRISTOBAL et al., Production of fuel ethanol from steam-explosion pretreated olive tree pruning. Fuel, 2008, Vol.87, pp. 692-700. See abstract, 2.2. pretreatment, and Table 1.	1-3, 10-14, 22

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 April 2013 (29.04.2013)

Date of mailing of the international search report

29 April 2013 (29.04.2013)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
189 Cheongsu-ro, Seo-gu, Daejeon Metropolitan
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Facsimile No. 82-42-472-7140

Authorized officer

KIM, Eun Hee

Telephone No. 82-42-481-5543



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/069854

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAHERZADEH , MONAMMAD J. et al., Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. Int. J. Mol. Sci. , 2008, Vol.9, pp.1621-1651. See abstract, 2.2 Dilute acid hydrolysis, and 3.1. Dilute acid pretreatment.	1-3,10-14,22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/069854**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-9,15-21
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2012/069854

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4,612,286 A	16 .09 . 1986	None	