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(54) **METHOD OF PRODUCING AN UPGRADED BIO-OIL**

(76) Inventors: **Phillip E. Savage**, Ann Arbor, MI (US); **Peigao Duan**, Jiaozuo (CN)

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(57) **ABSTRACT**

A method of producing an upgraded bio-oil from a wet biomass that includes heating the wet biomass at a first temperature and a first pressure for a time period ranging from 10 to 200 minutes to form a crude bio-oil. The first temperature ranges from 200 to 400° C. and the first pressure ranges from 0.1 to 25 MPa, with the proviso that at least a portion of the water present in the wet biomass remains in a liquid phase throughout the step of heating to form the crude bio-oil, and the first temperature and pressure are below super-critical conditions for water. The method also includes heating the crude bio-oil and the water at a second temperature and a second pressure to form the upgraded bio-oil. Some water remains in the liquid phase or in a super-critical fluid phase throughout the step of heating to form the upgraded bio-oil.

FIG. 1

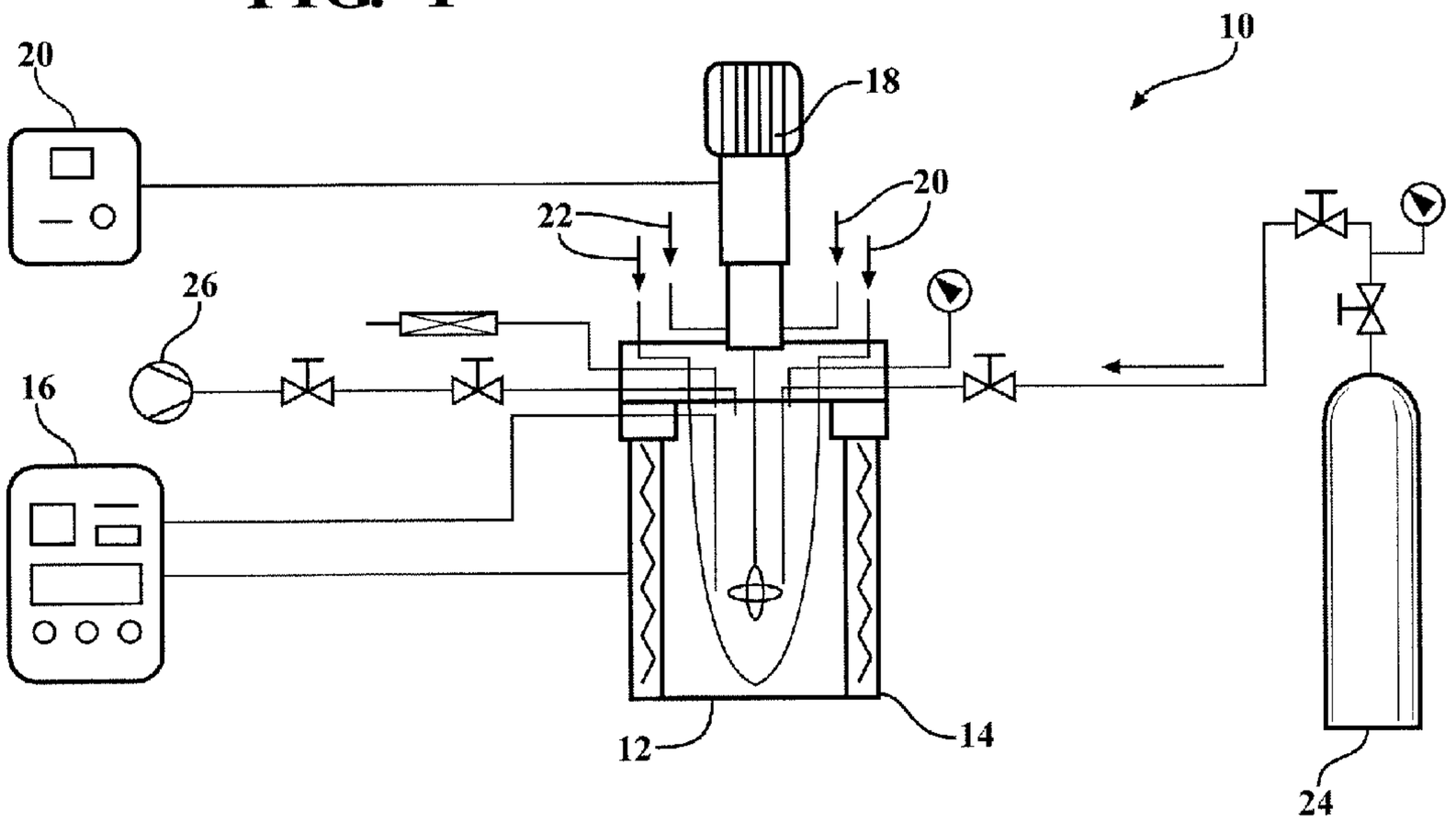
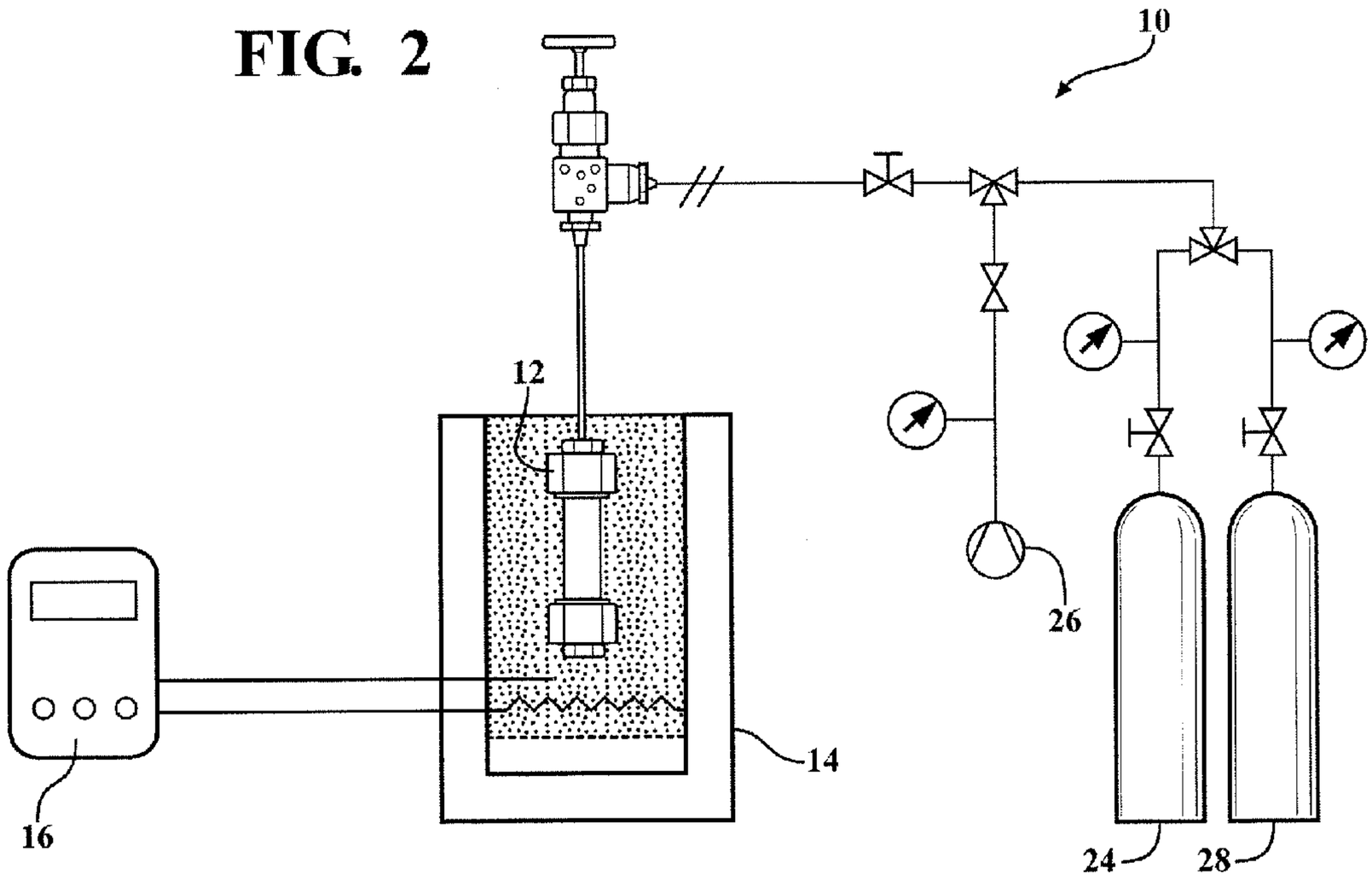


FIG. 2



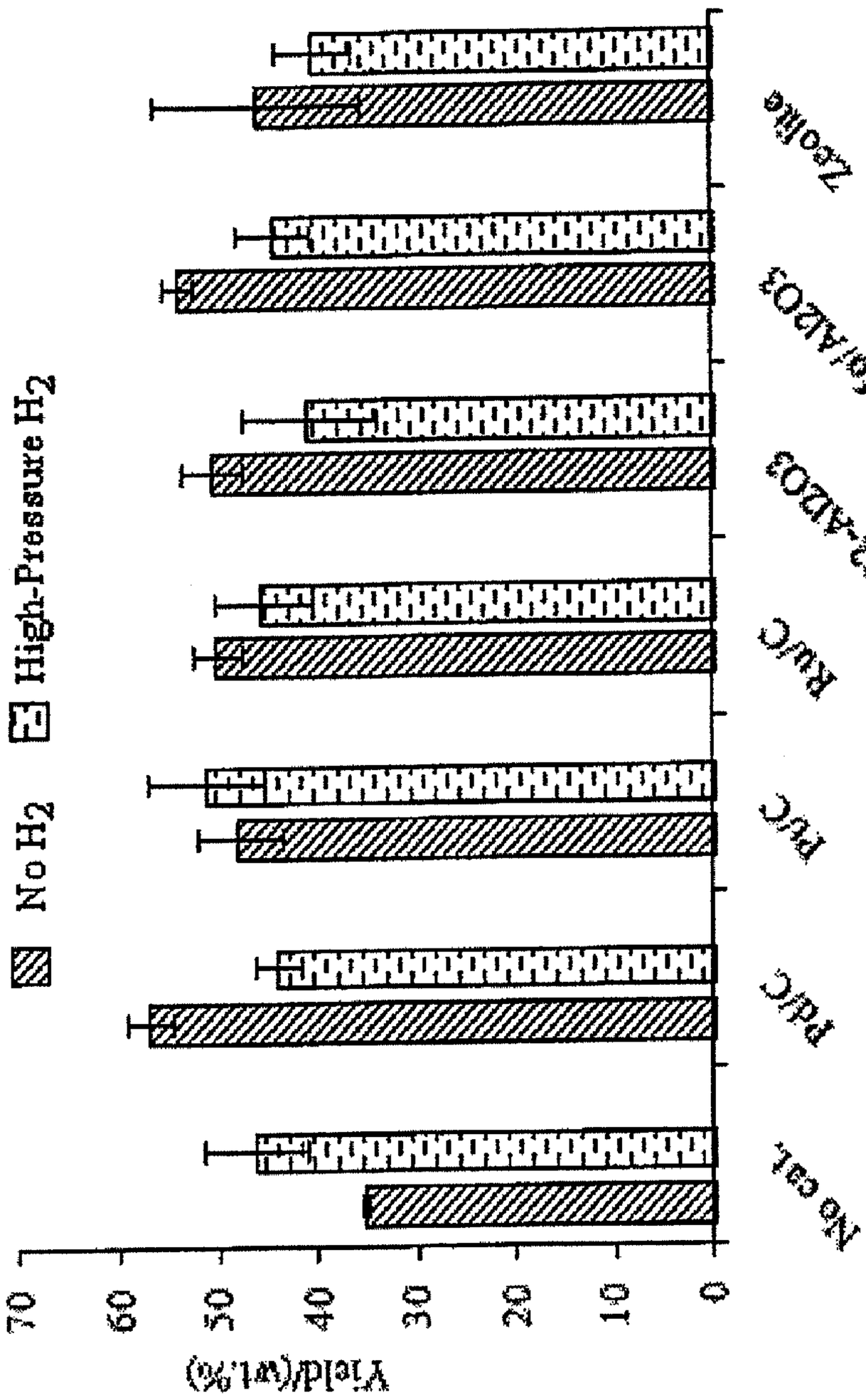

No H_2
High-Pressure H_2
Yield/(wt.%)
70
60
50
40
30
20
10
0
No cat.
Pd/C
Pd/C
Ru/C
Ru/C
Ni/SiO2-Al2O3
Ni/SiO2-Al2O3
Co/Mo/Al2O3
Co/Mo/Al2O3

FIGURE 3

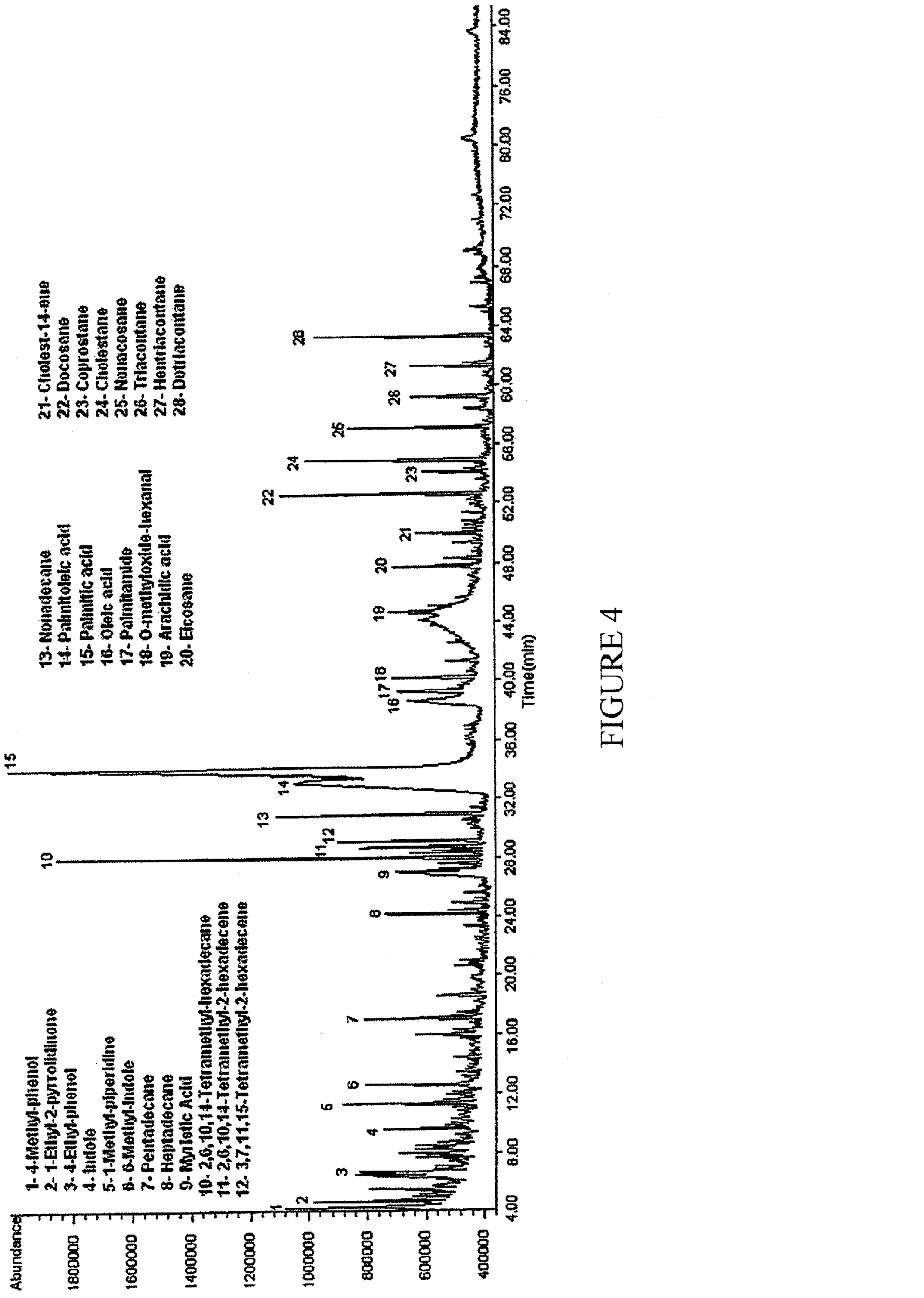

Abundance
1- 4-Methyl-phenol
2- 1-Ethyl-2-pyrrolidinone
3- 4-Ethyl-phenol
4- Indole
5- 1-Methyl-piperidine
6- 6-Methyl-Indole
7- Pentadecane
8- Heptadecane
9- Myristic Acid
10- 2,6,10,14-Tetramethyl-hexadecane
11- 2,6,10,14-Tetramethyl-2-hexadecene
12- 3,7,11,15-Tetramethyl-2-hexadecene
13- Nonadecane
14- Palmitoleic acid
15- Palmitic acid
16- Oleic acid
17- Palmitamide
18- O-methyloxide-hexanal
19- Arachidic acid
20- Eicosane
21- Cholest-14-ene
22- Docosane
23- Coprostane
24- Cholestane
25- Nonacosane
26- Triacontane
27- Hentriacontane
28- Dotriacontane
Time(min)

FIGURE 4

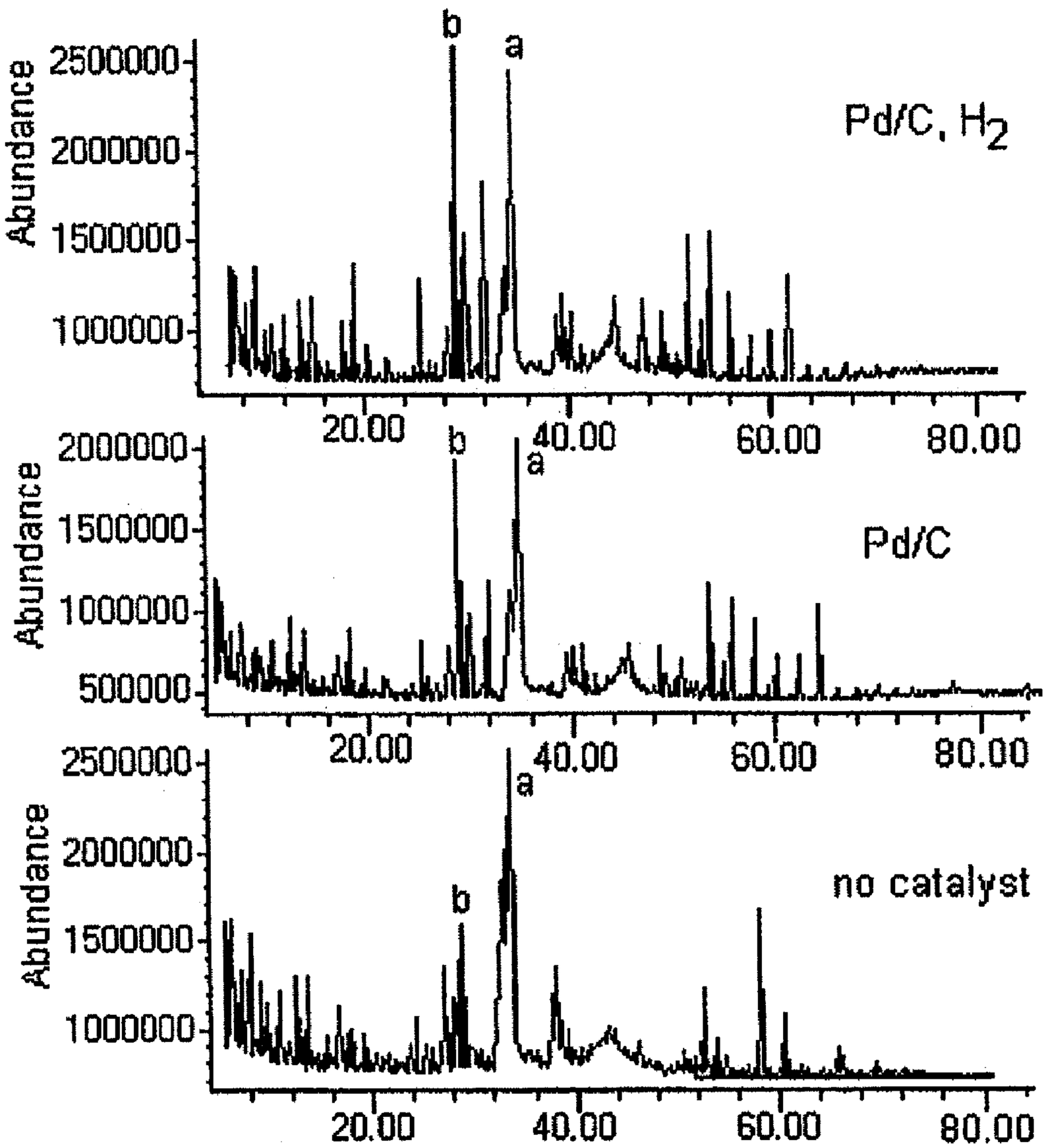

Abundance
2500000
2000000
1500000
1000000
20.00
40.00
60.00
80.00
b
a
Pd/C, H_2
Pd/C
500000
no catalyst

FIGURE 5

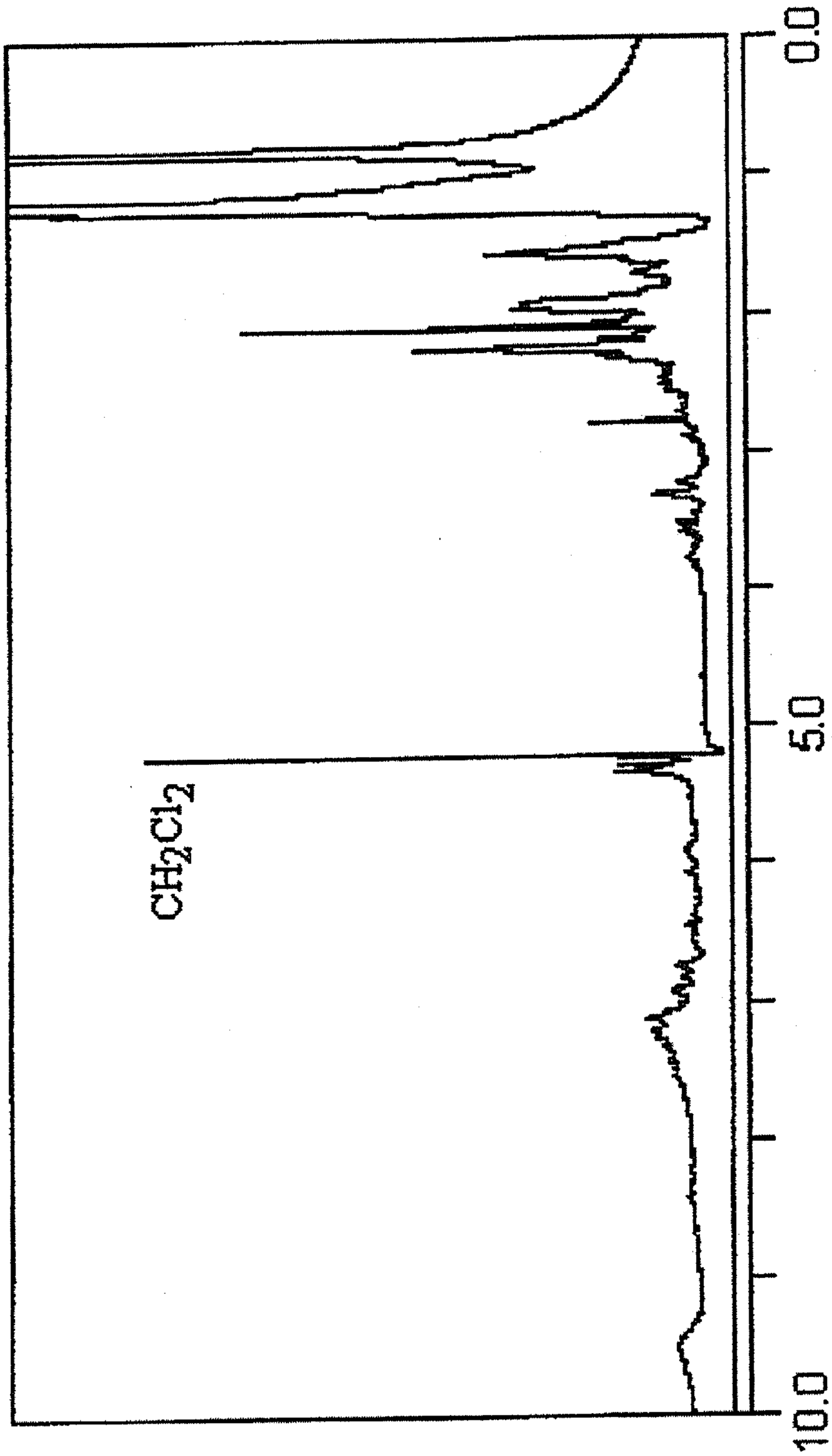


FIGURE 6

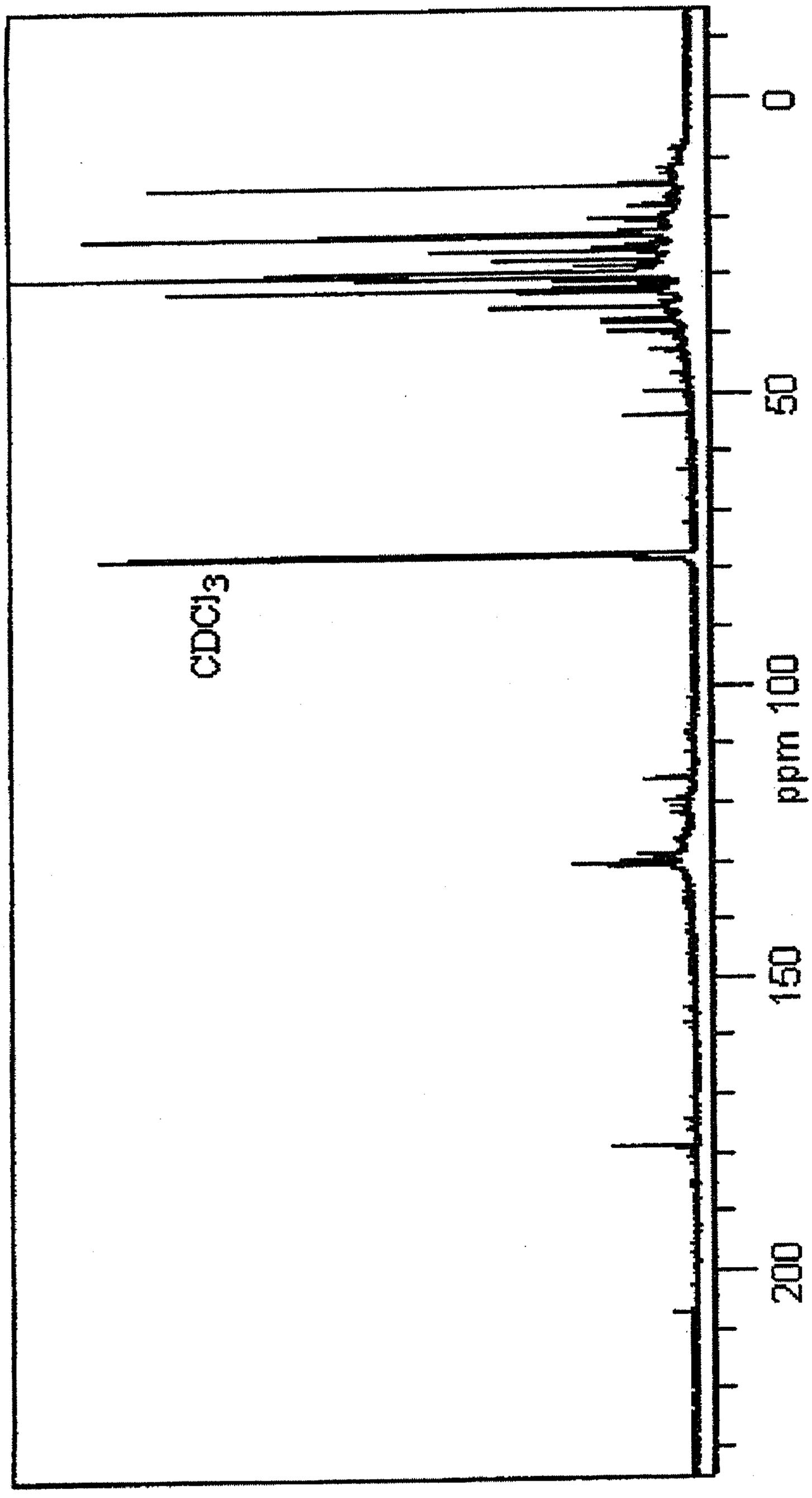


FIGURE 7

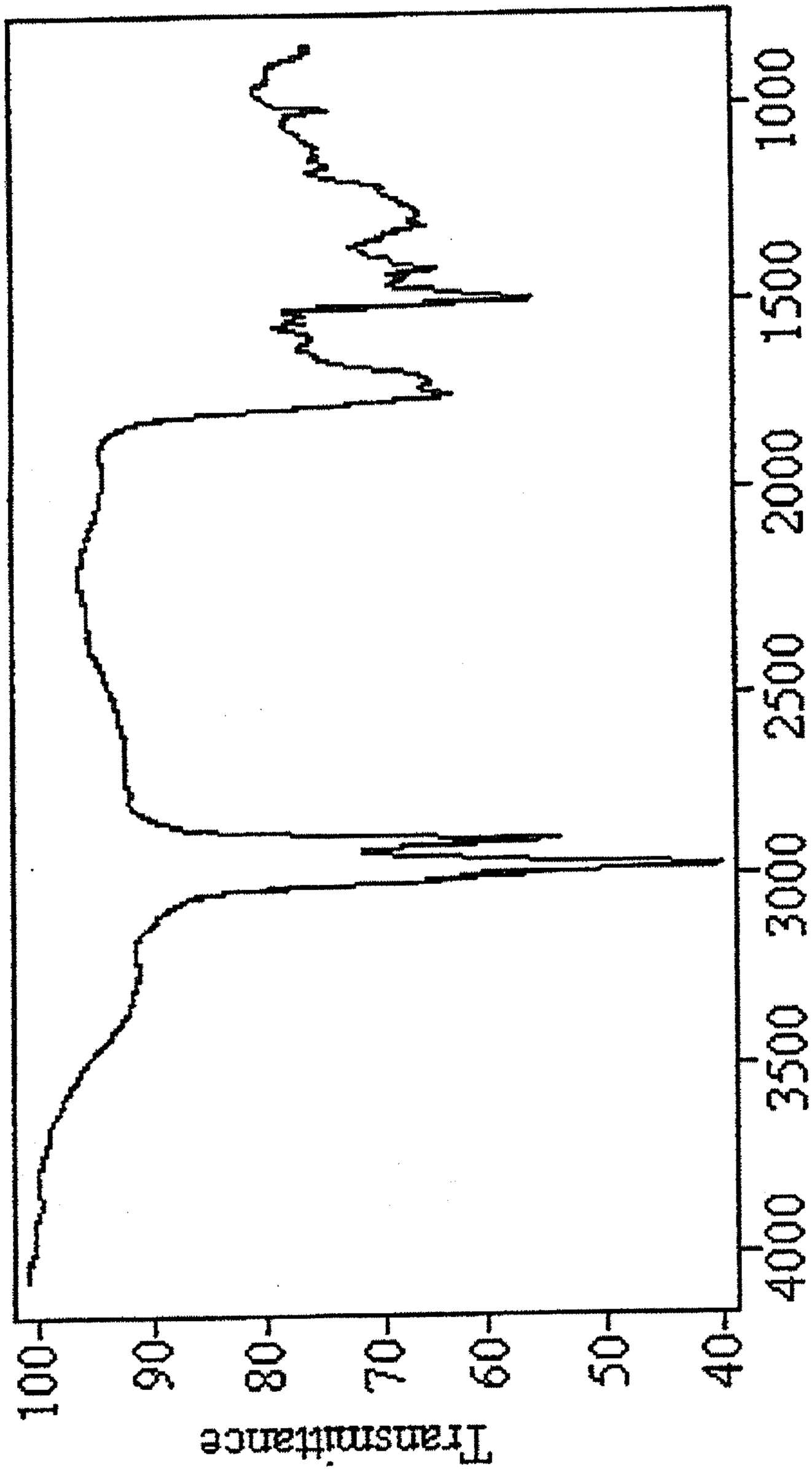

Transmittance
100
90
80
70
60
50
40
4000
3500
3000
2500
2000
1500
1000
Wavenumbers

FIGURE 8

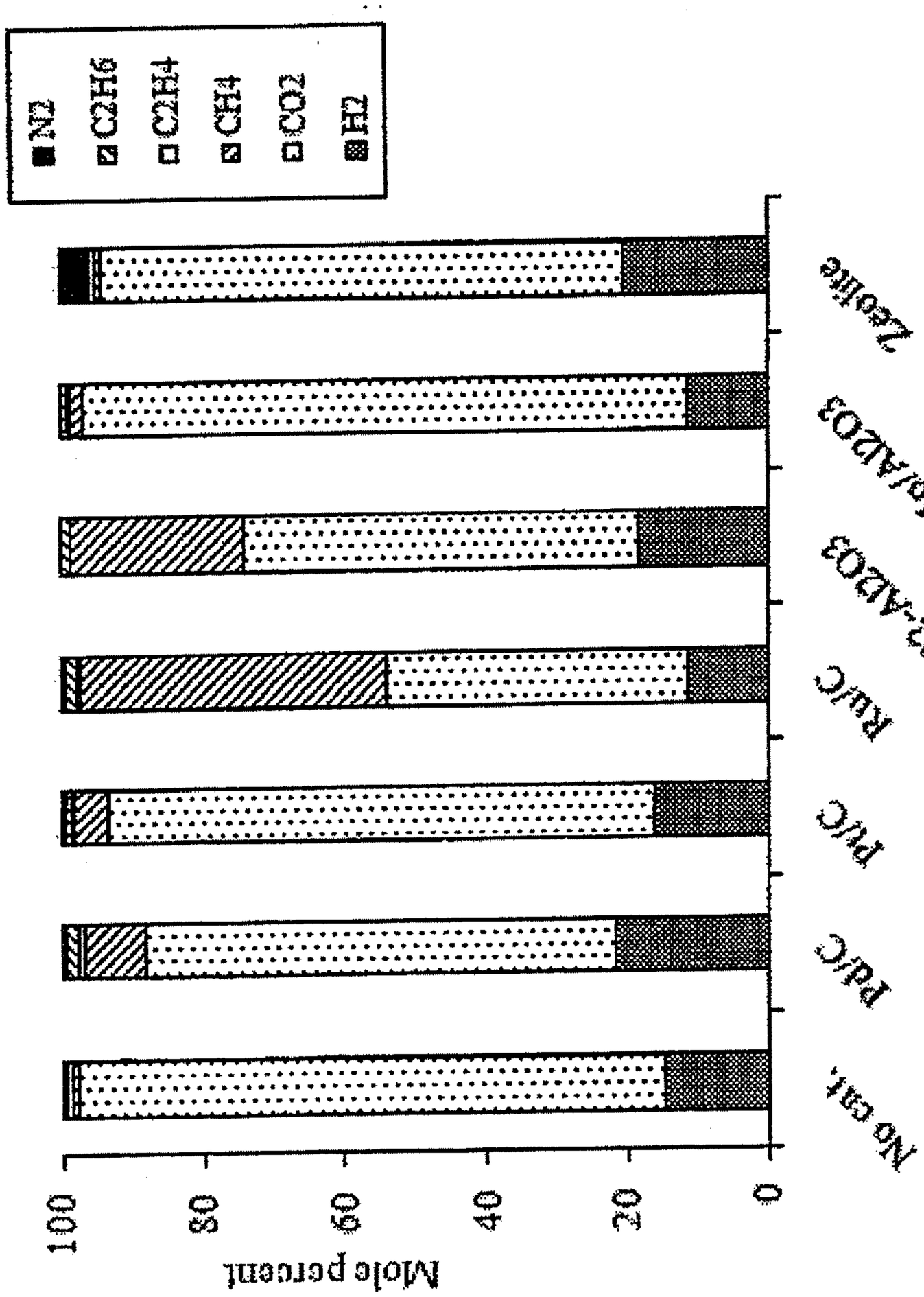

N2
C2H6
C2H4
CH4
CO2
H2
Zeolite
Co/Al2O3
Ni/SiO2-Al2O3
Ru/C
Pt/C
Pd/C
No cat.
100
80
60
40
20
0
Mole percent

FIGURE 9

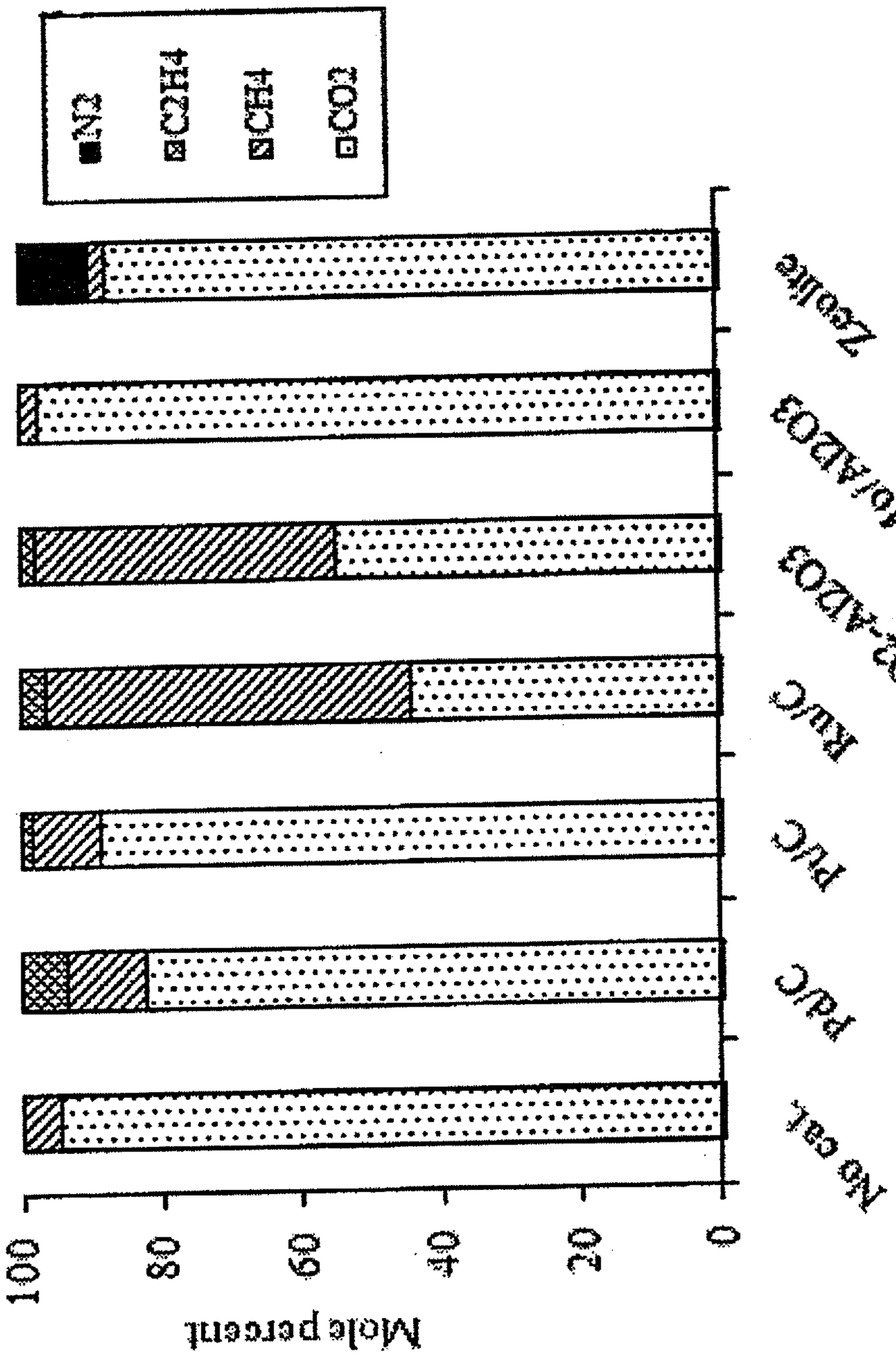

N2
C2H4
CH4
CO2
Pd/C
Pt/C
Ru/C
Ni5iO2-Al2O3
CoMo/Al2O3
Zeolite
Mole percent
100
80
60
40
20
0

FIGURE 10

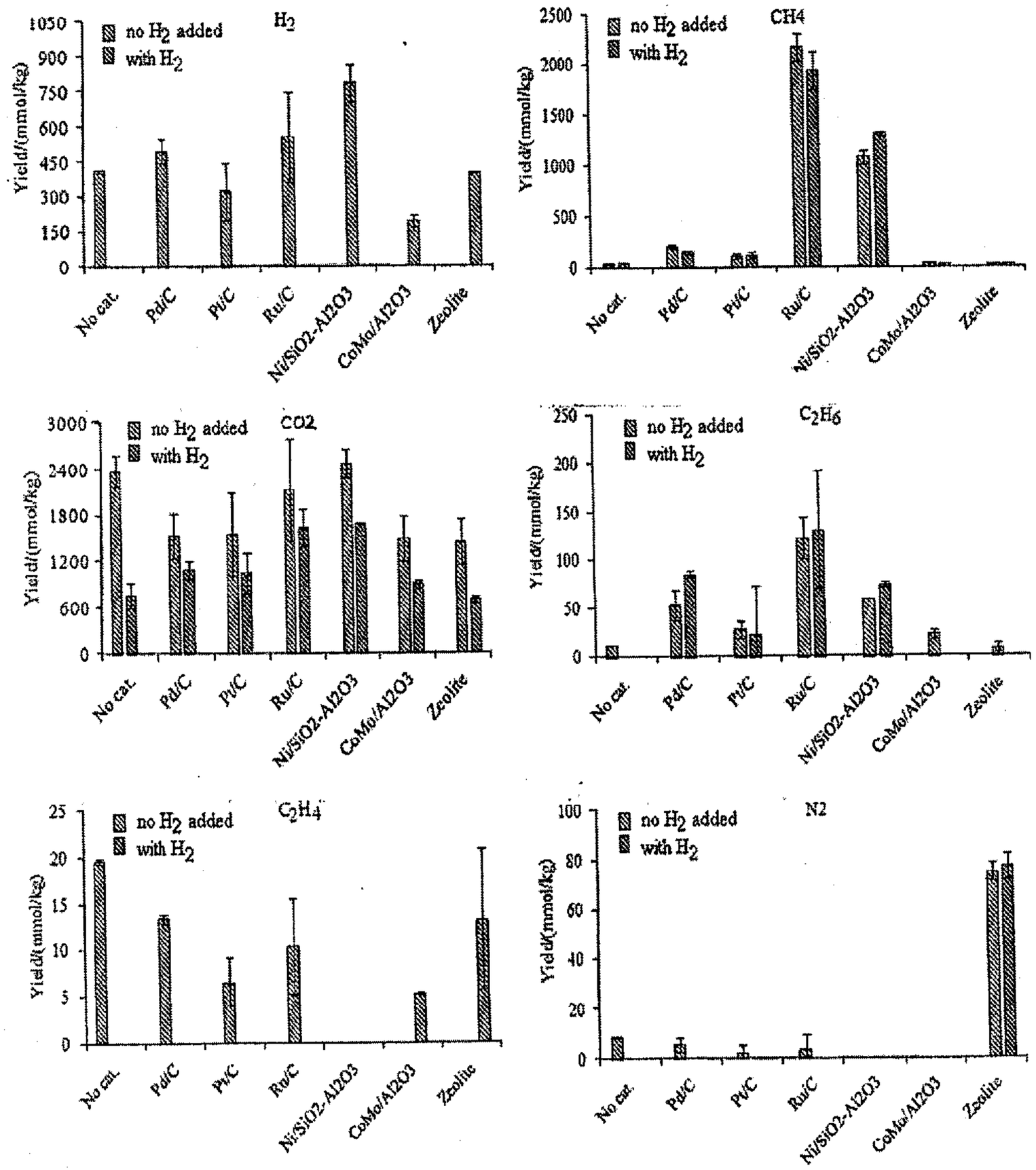

H2
no H2 added
with H2
Yield(mmol/kg)
1050
900
750
600
450
300
150
0
No cat.
Pd/C
Pt/C
Ru/C
Ni/SiO2-Al2O3
CoMo/Al2O3
Zeolite
CH4
2500
2000
1500
1000
500
CO2
3000
2400
1800
1200
600
C2H6
250
200
150
100
50
C2H4
25
20
15
10
5
N2
100
80
60
40

FIGURE 11

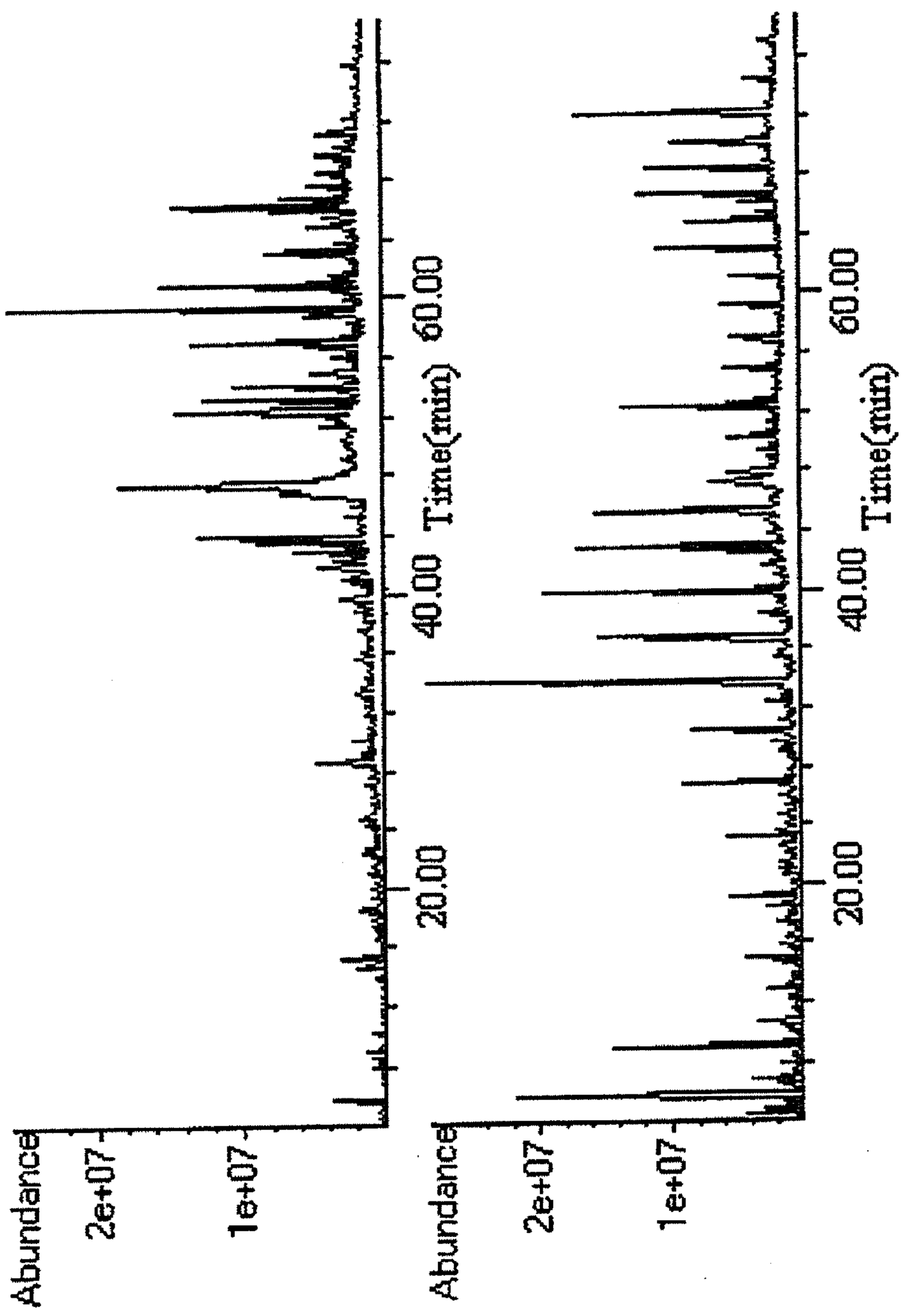

Abundance
2e+07
1e+07
20.00
40.00
60.00
Time(min)
Abundance
2e+07
1e+07
20.00
40.00
60.00
Time(min)

FIGURE 12

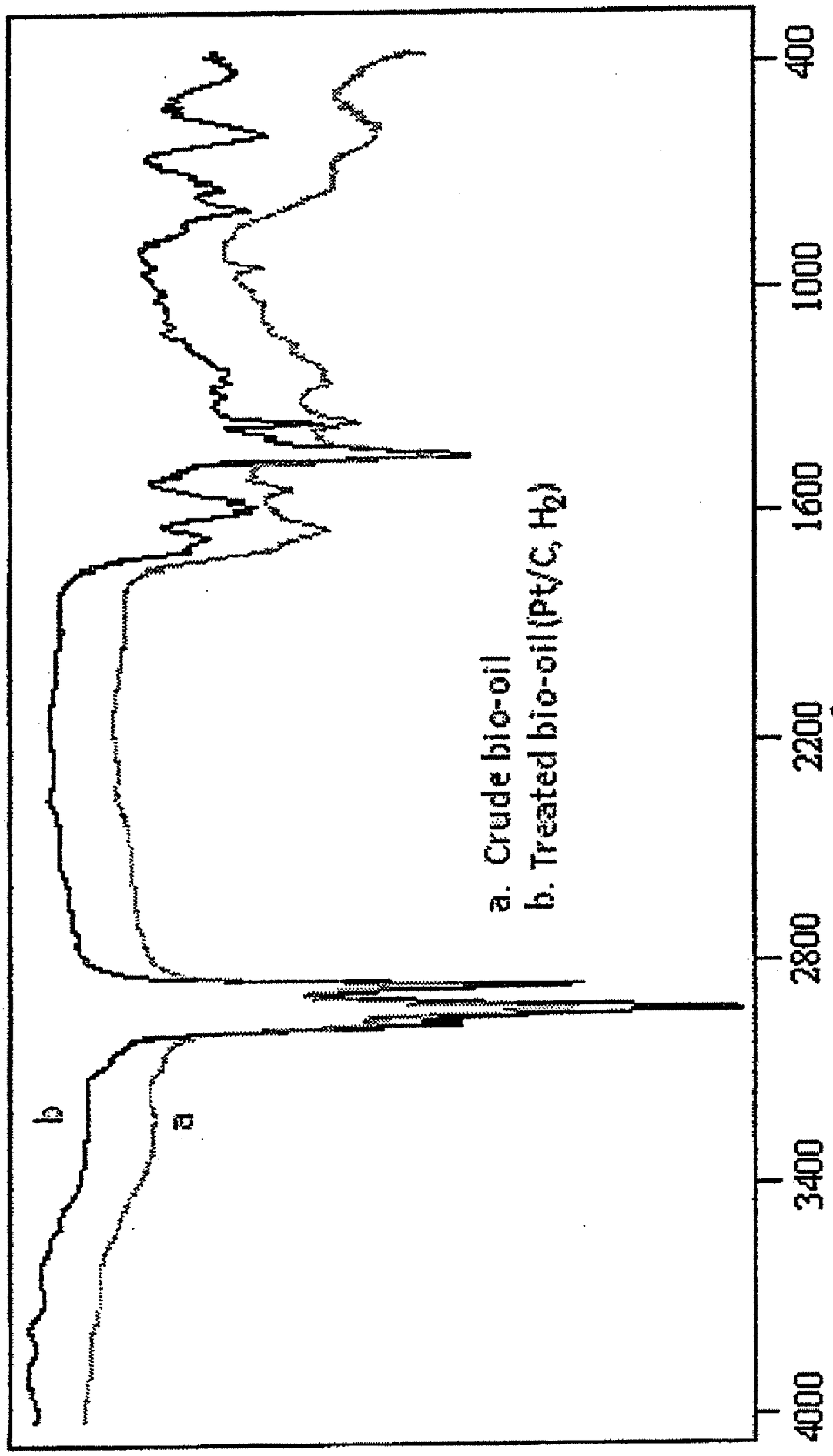

400
1000
1600
2200
2800
3400
4000
(cm-1)
a. Crude bio-oil
b. Treated bio-oil(Pt/C, H2)
b
a

FIGURE 13

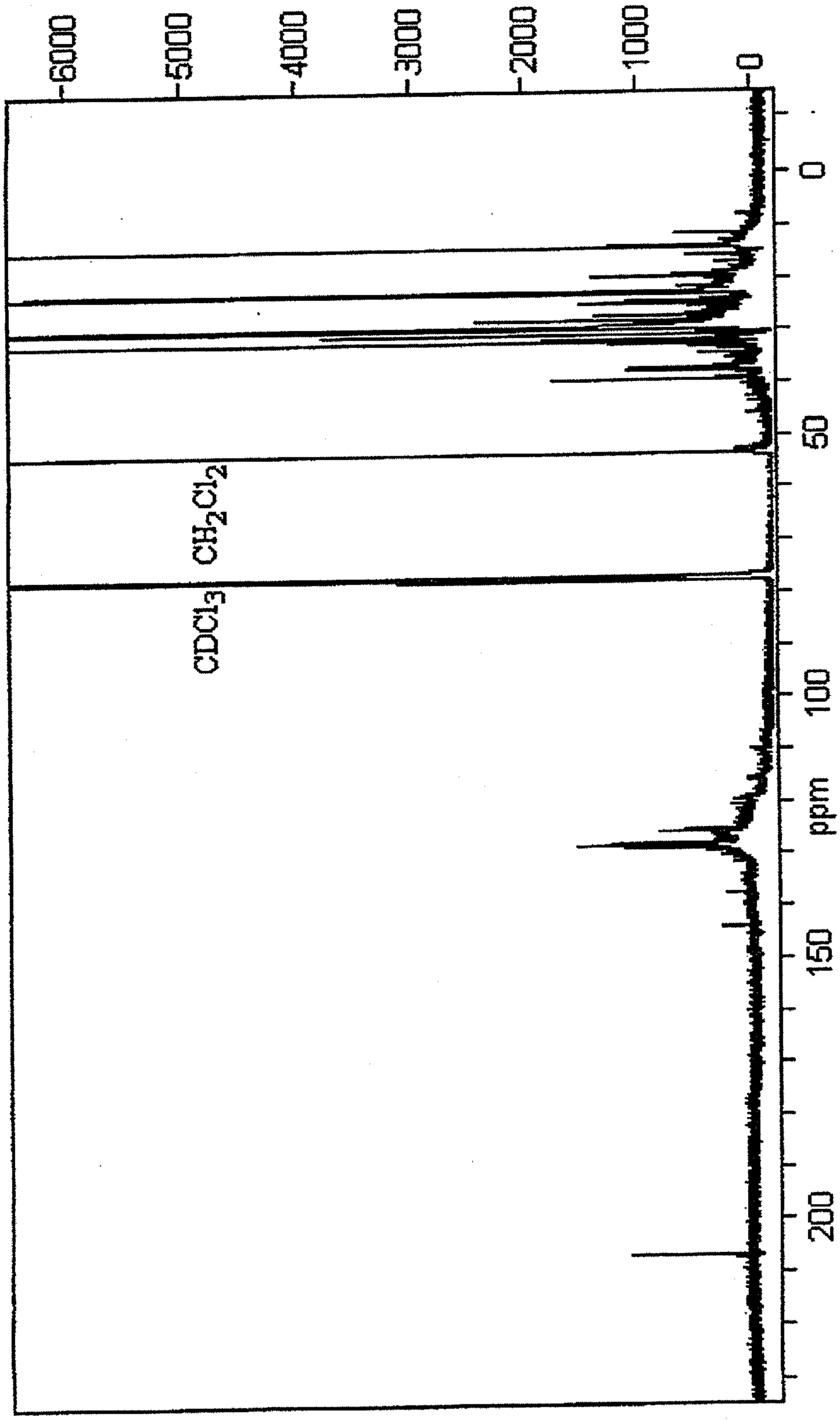

6000
5000
4000
3000
2000
1000
0
0
50
100
150
200
ppm
CH_2Cl_2
$CDCl_3$

FIGURE 14

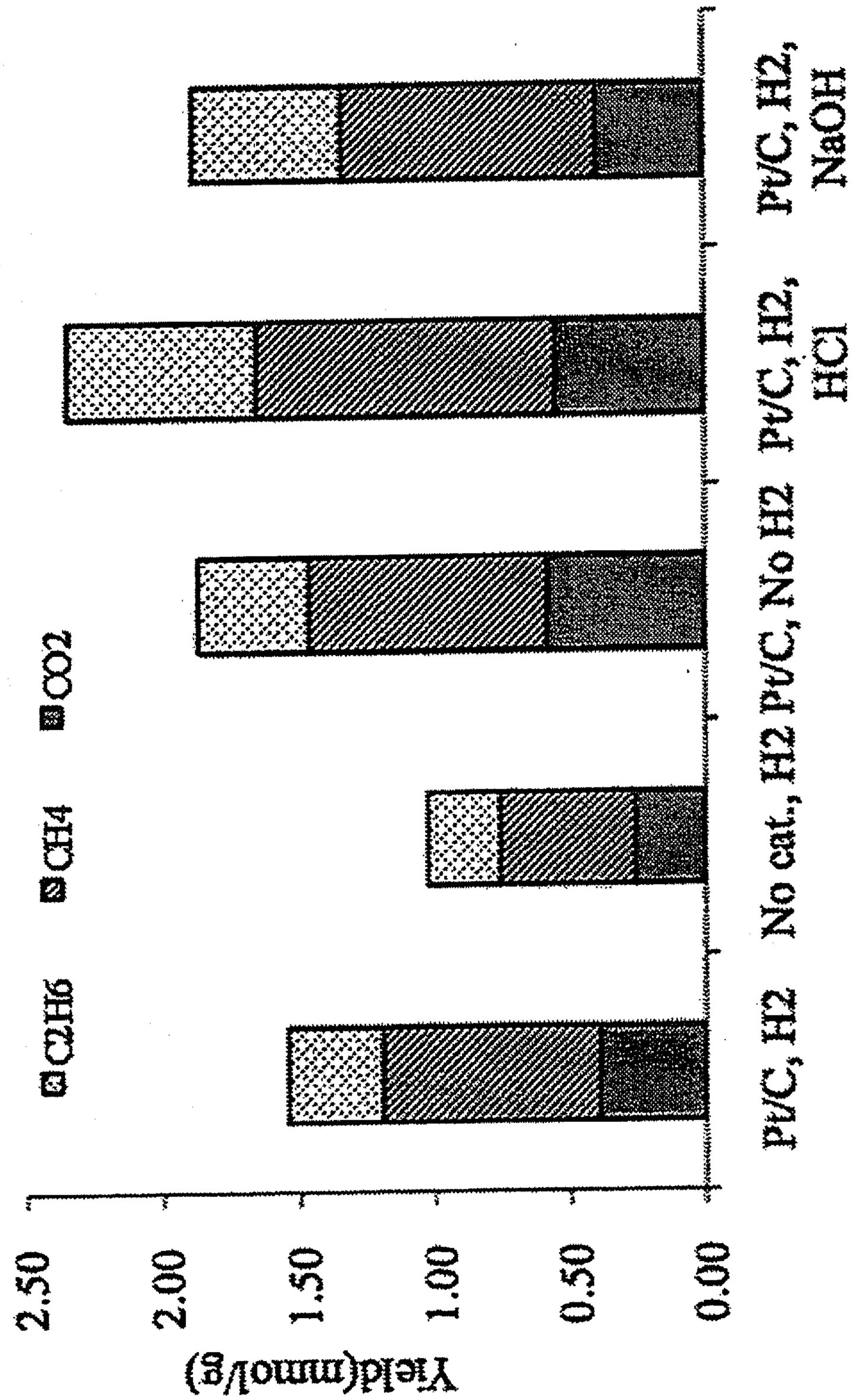

Pt/C, H2, NaOH
No cat., H2
Pt/C, No H2
Pt/C, H2, HCl
Pt/C, H2
CO2
CH4
C2H6
Yield(mmol/g)
2.50
2.00
1.50
1.00
0.50
0.00

FIGURE 15

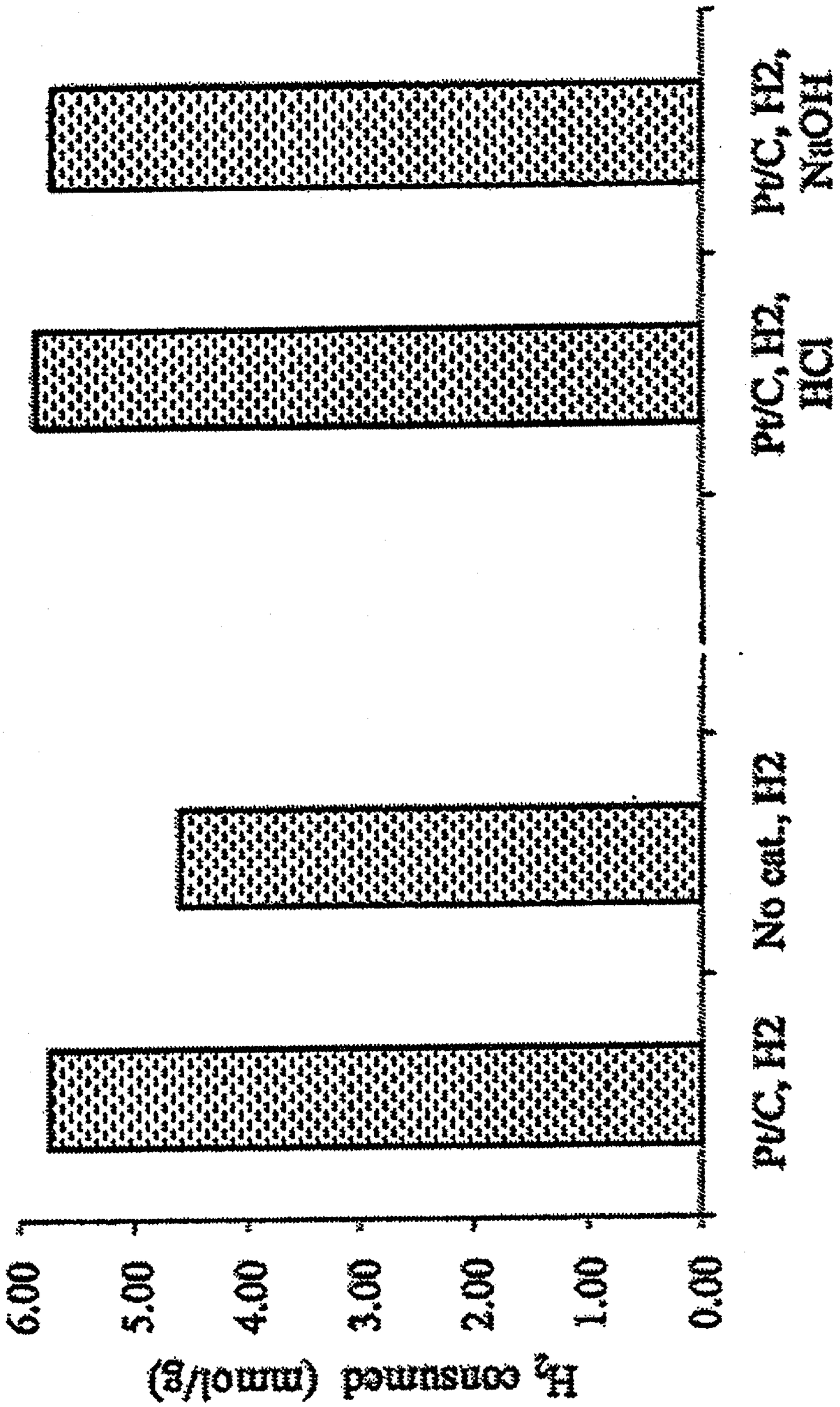

Pt/C, H2, NaOH
Pt/C, H2, HCl
No cat., H2
Pt/C, H2
0.00
1.00
2.00
3.00
4.00
5.00
6.00
H2 consumed (mmol/g)

FIGURE 16

METHOD OF PRODUCING AN UPGRADED BIO-OIL

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/379,723, filed on Sep. 2, 2010, the disclosure of which is incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT RIGHTS

[0002] This invention was made with government support under Grant No. EFRI-0937992 awarded by the National Science Foundation. The government may have certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention generally relates to a method of producing an upgraded bio-oil, specifically to a method of producing an upgraded bio-oil from a wet biomass.

BACKGROUND OF THE INVENTION

[0004] Worldwide energy demand, particularly for fossil fuels, is rising as populations increase and nations develop. Concurrently, concerns regarding climate change, declining fossil fuel reserves, and national security have moved to the forefront of society. These concerns have largely arisen due to unpredictable energy costs as well as a greater understanding of the effects of burning fossil fuel. Individuals and countries alike are searching for alternative energy sources which may supplement, or even replace, fossil fuels. Bio-oil is being explored as one possible alternative energy source.

[0005] Bio-oil is generally derived from a biomass, e.g., organisms and/or organic materials, which may be used to produce energy. Bio-oil is generally classified as either "first generation" bio-oil, "second generation" bio-oil, or "third generation" bio-oil. First generation bio-oil is produced from "edible" biomass such as corn, soybeans, or other crops. Generally, the edible biomass is processed in a manner which generates liquid bio-oil, such as grain ethanol and soy biodiesel. Second-generation bio-oils are produced from non-food biomass, such as lignocellulosic biomass, e.g., jatropha oil. Third-generation bio-oil is generally referred to as algae fuel and is derived from algae. Third-generation bio-oil is generally derived from lipids in the algae.

[0006] To date, two main processing strategies for bio-oil production from biomasses having moisture content ("wet biomass"), have been explored: 1) oil extraction with cell disruption and subsequent transesterification and 2) acid-catalyzed in-situ transesterification. While a variety of cell disruption techniques have been investigated for wet biomasses, most generally rely on organic solvents for oil recovery and expensive process operations that are difficult to implement on a large scale.

[0007] Although promising, bio-oil produced from wet biomass using the methods described above is generally not ready for use as a liquid transportation fuel. The bio-oil must undergo additional treatments to "refine" particular properties/characteristics of the bio-oil so that the bio-oil has comparable properties/characteristics to traditional fossil fuels. Furthermore, the processes for producing bio-oil and for upgrading the bio-oil generally require different reaction mediums, additional materials, processing steps, and increased production time.

[0008] Accordingly, there remains an opportunity to develop a method of producing an upgraded bio-oil from a wet biomass to reduce cost, reduce environmental impact, and increase production efficiencies.

SUMMARY OF THE INVENTION AND ADVANTAGES

[0009] A method of producing an upgraded bio-oil from a wet biomass is provided. The method includes providing the wet biomass comprising water and biomass solids and heating the wet biomass at a first temperature and a first pressure for a time period ranging from 10 to 200 minutes to form a crude bio-oil. The first temperature ranges from 200 to 400° C. and the first pressure ranges from 0.1 to 25 MPa, with the proviso that at least a portion of the water present in the wet biomass remains in a liquid phase throughout the step of heating to form the crude bio-oil, and the first temperature and first pressure are below super-critical conditions of water. The method also includes heating the crude bio-oil and the water at a second temperature and a second pressure for a time period of at least 30 minutes to form the upgraded bio-oil with the proviso that at least a portion of the water remains in a liquid phase or in a super-critical fluid phase throughout the step of heating to form the crude bio-oil. The second temperature is greater than the first temperature and is at least 300° C.

[0010] The method of producing upgraded bio-oil from the wet biomass as disclosed herein has many advantages. Namely, the method described herein involves aqueous phase processing, which results in reduced costs, reduced environmental impact, and reduced energy consumption. Because the wet biomass is heated at the first temperature and the first pressure, without vaporizing all of the water, utility costs are largely minimized. Because the wet biomass is heated at the first temperature and the first pressure in the presence of water, the costs of solvent are largely avoided. Furthermore, the upgraded bio-oil produced through heating the crude bio-oil and water at the second temperature and the second pressure has an excellent heating value and derived from a renewable resource.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Other advantages of the present invention will be readily appreciated, as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings.

[0012] FIG. **1** is a schematic of a biomass refinement system for heating a wet biomass in accordance with one embodiment.

[0013] FIG. **2** is a schematic of a biomass refinement system for heating crude bio-oil and water to form an upgraded bio-oil in accordance with another embodiment.

[0014] FIG. **3** is a bar graph illustrating the yield of crude bio-oil formed in the presence of different catalysts.

[0015] FIG. **4** is a total ion chromatogram of crude bio-oil formed from the step of heating the wet biomass at the first temperature and the first pressure in the presence of Pd/C first catalyst and an inert atmosphere.

[0016] FIG. **5** provides three total ion chromatograms of crude bio-oil formed from the step of heating the wet biomass at the first temperature and the first pressure under varying conditions.

[0017] FIG. **6** is a ^{1}H NMR spectrum of crude bio-oil in chloroform formed from the step of heating the wet biomass at the first temperature and the first pressure in the presence of a Pd/C first catalyst and the inert atmosphere.

[0018] FIG. **7** is a ^{13}C NMR spectrum of crude bio-oil in chloroform formed from the step of heating the wet biomass at the first temperature and the first pressure in the presence of a Pd/C first catalyst and the inert atmosphere.

[0019] FIG. **8** is a FT-IR spectrum of crude bio-oil formed from the step of heating the wet biomass at the first temperature and the first pressure in the presence of a Pd/C first catalyst in an inert atmosphere.

[0020] FIG. **9** is a bar graph illustrating effects of heating the wet biomass at the first temperature and the first pressure in the presence of different first catalysts in an inert atmosphere.

[0021] FIG. **10** is a bar graph illustrating effects of heating wet biomass at the first temperature and the first pressure in the presence of different first catalysts in a reducing atmosphere.

[0022] FIG. **11** provides a plurality of bar graphs, each illustrating the yield of the gas above the crude bio-oil from the step of heating wet biomass at the first temperature and the first pressure in the presence of a first catalyst, a reducing atmosphere, and an inert atmosphere.

[0023] FIG. **12** provides two total ion chromatograms: the top graph shows the effect of heating wet biomass at the first pressure and the first temperature in a reducing atmosphere; and the bottom graph shows the effect of heating crude bio-oil and water at the second temperature and the second pressure in the presence of a Pt/C second catalyst and a reducing atmosphere.

[0024] FIG. **13** is a FT-IR spectrum of crude bio-oil and upgraded bio-oil formed by heating crude bio-oil and water at the second temperature and the second pressure in the presence of a Pt/C second catalyst and a reducing atmosphere.

[0025] FIG. **14** is a ^{13}C NMR spectrum of the upgraded bio-oil formed by the step of heating crude bio-oil and water at the second temperature and the second pressure in the presence of a Pt/C second catalyst and a reducing atmosphere.

[0026] FIG. **15** is a bar graph illustrating yields of upgraded bio-oil resulting from the step of heating crude bio-oil at the second temperature and the second pressure under different conditions.

[0027] FIG. **16** is a bar graph illustrating the distribution of gas products resulting from the step of heating the crude bio-oil at the second temperature and the second pressure under varying conditions.

DETAILED DESCRIPTION OF THE INVENTION

[0028] A method of producing upgraded bio-oil from a wet biomass is provided. The method includes providing the wet biomass comprising water and biomass solids. The wet biomass contains biomass solids in an amount ranging from 1 to 30 wt. %, alternatively, from 1 to 20 wt. %; alternatively, from 1 to 10 wt. %, all based on the total weight of the wet biomass. Typically, water is present in the wet biomass in an amount ranging from 70 to 99 wt. %, alternatively, from 80 to 99 wt. %, or, alternatively, from 90 to 99 wt. %, all based on the total weight of the wet biomass. Alternatively, water may be present in the wet biomass in an amount ranging from 90 to 95 wt. %, based on the total weight of the wet biomass.

[0029] Typically, the biomass solids include organisms, e.g., multicellular organisms, single-celled organisms, cell fragments/components of the multicellular or single celled organisms, e.g., organelles, proteins, lipids, and the like. Suitable examples of biomass solids are derived from various forms of algae. A specific example of algae includes, but is not limited to, *Nannochloropsis* sp, which may be obtained from Reed Mariculture, as Nannochloropsis 3600™. Alternatively, the algae may comprise *Chlorella vulgaris*. However, it is to be appreciated that various different types of biomass solids can be used based off of selection criteria including, but not limited to, lipid yields, fatty acid profile, growth/reproduction rate, photosynthetic efficiency, and combinations thereof.

[0030] The method may include the step of producing biomass solids. The biomass solids can be produced through growing algae. Typically, the biomass solids are grown in at least one bioreactor. Suitable examples of the bioreactor include, but are not limited to, continuously-fed bubble column reactors and stir tanks. The biomass solids may be grown phototrophically and/or heterotrophically. In another embodiment, the biomass solids is grown phototrophically in a series of continuously-fed bubble column reactors and then heterotrophically in a stir tank. Without being bound to any particular theory, it is believed that sequential phototrophic and heterotrophic growth of the biomass solids increases biomass density, lipid productivity, lipid content, lipid profile, fatty acid content, and carbon substrate utilization efficiency of the biomass solids.

[0031] The step of growing the biomass solids may include treating the biomass solids to maximize lipid productivity and/or biomass solids density. Typically, the biomass solids are treated by chemical and/or physical stimulation. Suitable examples of chemical and/or physical stimulation include, but are not limited to, manipulating nutrient concentration, pH, temperature, irradiance, genes, and combinations thereof. In one embodiment, the biomass solids are subjected to nitrogen stress. In another embodiment, the biomass solids are grown on glucose and in heterotrophic growth conditions for a period of time. In this embodiment, the period of time typically ranges from 1 to 10 days, or, alternatively, from 4 to 8 days, or, alternatively, from 6 to 8 days. Without being bound to any particular theory, it is believed that treating the biomass solids with nitrogen stress in heterotrophic growth conditions also improves lipid productivity of the biomass solids.

[0032] In one or more embodiments, the method may further include the step of dewatering the biomass solids. Suitable examples of dewatering techniques include, but are not limited to, centrifugation, gravity sedimentation, autoflocculation, flocculation with organic or microbial products, in-situ microbial flocculation, dissolved air flotation, belt filtration, membrane filtration, and combinations thereof. Dewatering techniques may be used to further improve the density of the biomass solids. However, it is to be appreciated that the step of dewatering is optional.

[0033] The biomass solids may be prepared from an algae paste and subsequently mixed with water to form the wet biomass. Alternatively, the biomass solids may be prepared raw, i.e., the source of biomass solids may be used without dewatering.

[0034] The lipid content of the biomass solids is typically measured as a percentage by weight of all lipids present in the

biomass solids, based on the total weight of the biomass solids. In one embodiment, the biomass solids have a lipid content ranging from 20 to 80 wt. %, or, alternatively, from 30 to 60 wt. %, all based on the total weight of the biomass solids.

[0035] The method disclosed herein may be performed in a variety of devices, as will be appreciated by one of ordinary skill in the art. Referring to FIG. **1**, in one embodiment, the method may be performed in a biomass refinement system **10**. The heating system may include a reaction vessel **12**. The reaction vessel **12** may be used as part of a crude bio-oil reactor or as part of a upgrading reactor. The reaction vessel **12** has a design and configuration to withstand the temperature and pressure conditions of the method disclosed herein. In one embodiment, the reaction vessel **12** comprises an autoclave. The reaction vessel **12** may be heated with a heating system **14**. The heating system **14** may comprise a heating jacket that contacts the reaction vessel **12**. The heating jacket **14** may be used in conjunction with other components that may be present in the heating system, such as a thermal sand bath. Alternatively, the reaction vessel **12** may be heated with a fluidized sand bath (see FIG. **2**). In other embodiments, the reaction vessel **12** may be connected to other devices sufficient to provide isothermal heating. The biomass refinement system **10** may also include a temperature controller **16**, operative to control the temperature within the reaction vessel **12**.

[0036] The biomass refinement system **10** may also include a mixer device **18** and a mixer controller **20**. The mixer device **18** may be selected from various paddles, stirrers, and/or agitators, etc. as will be appreciated by one having ordinary skill in the art. The mixer device **18** may serve to mix the wet biomass throughout the step of heating the wet biomass at the first temperature and the first pressure. The mixer device **18** may also serve to mix the wet biomass throughout the step of heating the crude bio-oil and water at the second temperature and the second pressure. The mixer controller **20** may be coupled to the mixer device **18** and control the mixing speed of the mixer device **18**.

[0037] The biomass refinement system **10** may also include various input and output streams. In one embodiment, the biomass refinement system **10** may comprise at least one water inlet **20** and at least one water outlet **22** connected to the interior of the reaction vessel **12**. The biomass refinement system **10** may also include an H_2 system **24** connected to the reaction vessel **12** to provide pressurized hydrogen to the interior of the reaction vessel.

[0038] Referring to FIG. **2**, in another embodiment, the biomass refinement system **10** includes a different reaction vessel **12** with a heating system **14**. The heating system **14** may comprise a thermal sand bath in this second embodiment. The biomass refinement system **10** may include a vacuum pump **26** coupled to the interior of the reaction vessel **12** to remove air. The biomass refinement system may also include the H_2 system **24** and an inert gas system **28** which are connected to the interior of the reaction vessel.

[0039] It is also contemplated that the biomass refinement system **10** may include two or more reaction vessels. For example, in one embodiment, the biomass refinement system **10** could include a crude bio-oil reactor to heat the wet biomass at the first temperature and the first pressure to form the crude bio-oil. The biomass refinement system **10** could also include a upgrading reactor to heat the crude bio-oil and water at the second temperature and second pressure to form the upgraded bio-oil.

[0040] The method comprises heating the wet biomass at the first temperature and the first pressure for a time period ranging from 10 to 200 minutes to form the crude bio-oil. The first temperature ranges from 200 to 400° C. and the first pressure ranges from 0.1 to 25 MPa, with the proviso that at least a portion of the water present in the wet biomass remains in a liquid phase throughout the step of heating to form the crude bio-oil, and the first temperature and first pressure are below super-critical conditions of water. The first temperature and the first pressure may also be sufficient to provide superheated water.

[0041] By "liquid phase," it is meant water that is not in a gaseous or vapor phase and water that is not a super-critical fluid.

[0042] The step of heating the wet biomass at the first pressure and the first temperature may be considered, in part, hydrothermal liquefaction. Hydrothermal liquefaction is the aqueous-phase conversion of the wet biomass into crude bio-oil. Generally, hydrothermal liquefaction converts the wet biomass into an oily or tarry fluid via reactions in and with liquid water at elevated temperatures and above the saturation pressure of water. In such a reaction, water may serve as solvent, catalyst (or catalyst precursor), and/or reactant (e.g., in hydrolysis reactions). The various components of the biomass solids are converted from carbonaceous solids to various hydrocarbons, phenolic compounds, and other fluids during hydrothermal liquefaction which may have heating value.

[0043] The temperature present in the reaction vessel during the step of heating the wet biomass is referred to as the first temperature. Typically, the first temperature is present in the reaction vessel throughout the step of heating the wet biomass to form the crude bio-oil. The first temperature may range from 250 to 400° C.; from 300 to 375° C.; or, alternatively, from 345 to 355° C. Other first temperatures are also contemplated, so long as at least a portion of the water present in the wet biomass is in the liquid phase throughout the step of heating to form the crude bio-oil and the first temperature and first pressure are below super-critical conditions.

[0044] A starting pressure of the reaction vessel, before the step of heating to the first pressure and the first temperature, may be increased with various mediums. In one embodiment, the wet biomass is pressurized at a pressure ranging from 10 to 4,000 kPa, or, alternatively, from 50 to 3,800 kPa, or 70 to 3,500 kPa with various gases, before the step of heating the wet biomass at the first temperature and the first pressure. The various gases may include, but are not limited to, helium and/or hydrogen.

[0045] As one of ordinary skill in the art will appreciate, by heating the wet biomass at the first temperature in the reaction vessel, the pressure in the reaction vessel will naturally rise. The pressure may increase beyond what is provided by the presence of the reducing atmosphere or the inert atmosphere. The result of this pressure increase is predictable through means of one of ordinary skill in the art. In one possible configuration, the density within the reaction vessel can be monitored in order to indirectly ascertain the pressure within the reaction vessel throughout the step of heating the wet biomass at the first temperature and the first pressure.

[0046] During the step of heating the wet biomass at the first temperature, an elevated pressure may be present within the reaction vessel. The pressure present in the reaction vessel during the step of heating the wet biomass at the first temperature is referred to as the first pressure. Typically, the first pressure is present throughout the step of heating the wet

biomass at the first temperature and the first pressure. The first pressure may range from 0.1 to 25 MPa, or, alternatively, from 0.1 to 20 MPa, or, alternatively, from 1 MPa to 10 MPa. Other first pressures are also contemplated, with the proviso that at least a portion of the water present in the wet biomass remains in a liquid phase throughout the step of heating to form the crude bio-oil, and the first temperature and first pressure are below super-critical conditions of water.

[0047] For the instant method, the wet biomass may be heated at the first temperature and the first pressure for a time period ranging from 10 to 200 minutes, or, alternatively, from 55 to 100 minutes, or, alternatively, from 55 to 70 minutes. Alternatively, the wet biomass may be heated at the first temperature and the first pressure for a time period of at least 30 minutes, up to 8 hours. Such time periods are sufficient to convert at lest a portion of the wet biomass into crude bio-oil. Other time periods are also contemplated, as one of ordinary skill in the art may recognize that the size and design of the reaction vessel and the amount of reactants may necessitate longer or shorter time periods.

[0048] In one embodiment, the step of heating the wet biomass at the first temperature and the first pressure is conducted in the presence of a first catalyst. Without intending to be bound by any particular theory, it is believed that using Pd/C, $Ni/SiO_2—Al_2O_3$, Ru/C, or zeolite as the first catalyst maximizes the mole fraction of H_2 produced in the crude bio-oil. Also, and without intending to be bound by any particular theory, it is believed that using Pd/C, Pt/C, Ru/C, or $Ni/SiO_2—Al_2O_3$ as the first catalyst maximizes the mole fraction of methane produced in the crude bio-oil. Without intending to be bound by any particular theory, it is believed that the $Ni/SiO_2—Al_2O_3$ catalyst has a desulfurization activity. Also, and without intending to be bound by any particular theory, it is believed that noble metal catalysts including, but not limited to, Pd/C, Pt/C, Ru/C, maximize the hydrogen to carbon molar ratio of the crude bio-oil. In the absence of a reducing atmosphere, i.e., pressurized hydrogen, all of the first catalysts tested produced higher yields of crude bio-oil when present during the heating of the wet biomass. However, elemental compositions and heating values of the crude bio-oil were largely insensitive to the catalyst used. It is also theorized that Ru/C and $Ni/SiO_2—Al_2O_3$ are capable of providing in situ denitrogenation during the step of heating of the wet biomass at the first temperature and the first pressure.

[0049] The color and apparent viscosity of the crude bio-oil may vary depending on the catalyst type. For example, the crude bio-oils obtained with Pd/C, Pt/C, Ru/C and $CoMo/\gamma\text{-}Al_2O_3$ flowed easily and were much less viscous than the crude bio-oil obtained with uncatalyzed or zeolite-catalyzed heating. The crude bio-oil formed from heating with the $Ni/SiO_2—Al_2O_3$ first catalyst had a dark red color.

[0050] The amount of first catalyst may range in an amount ranging from 5 to 75 wt. % or, alternatively, from 10 to 50 wt. %, or, alternatively, from 10 to 40 wt. %, or, alternatively, from 20 to 30 wt. %, all based on the total weight of the wet biomass. The first catalyst may comprise a heterogeneous catalyst selected from the group consisting of Pd/C, Pt/C, Ru/C, $Ni/SiO_2—Al_2O_3$, sulfided $CoMo/\gamma\text{-}Al_2O_3$, zeolite, activated carbon, and combinations thereof. Catalysts including Pd/C, Pt/C, Ru/C, $Ni/SiO_2\text{-}Al_2O_3$, and zeolite, are available from Sigma-Aldrich. $CoMo/\gamma\text{-}Al_2O_3$ (sulfided) was obtained from Alfa Aesar.

[0051] In one or more configurations, the step of the heating the wet biomass at the first temperature and the first pressure may be conducted in the presence of various additives including, but not limited to, metal salts and bases. The metal salts include, but are not limited to, NaCl, $MnCl_2$, $ZnCl_2$, $CoCl_2$, $CuSO_4$, and $MgSO_4$. The bases include, but are not limited to, NaOH and KOH. Without being limited by theory, it is believed that the additions of metal salts and/or bases may affect the extent of decarboxylation of the crude bio-oil.

[0052] In another embodiment, the step of heating the wet biomass at the first temperature and first pressure is conducted in a reducing atmosphere. It is believed that heating the wet biomass in the reducing atmosphere yields reduced amounts of CO_2 in the gas above the crude bio-oil and eliminates production of C_2H_4, which results in a higher quality crude bio-oil. The reducing atmosphere may be present in the reaction vessel before the step of heating the wet biomass. The presence of either the hydrogen or the higher pressure in the reaction system suppresses formation of gas. The total yield of the gas above the crude bio-oil is lower in a reducing atmosphere than in analogous conditions without the reducing atmosphere and at lower pressure.

[0053] In one embodiment, the reducing atmosphere comprises pressurized hydrogen gas. However, it is also contemplated that the reducing atmosphere may comprise other gaseous reducing agents. The pressurized hydrogen gas may be provided at a pressure ranging from 0.1 to 30 MPa. In other embodiments, the hydrogen gas may be provided at a pressure ranging from 20 to 30 MPa, or, alternatively, from 5 to 25 MPa. Without being limited by theory, it is believed that the pressurized hydrogen may hydrogenate the unsaturated heterocyclic ring and hydrocrack the subsequent products during the step of heating the wet biomass.

[0054] In yet another embodiment, the step of heating the wet biomass is conducted in the presence of the reducing atmosphere and the first catalyst. Without intending to be bound by any particular theory, it is believed that heating the wet biomass in the presence of the catalyst and in the reducing atmosphere maximizes the hydrogen content and hydrogen to carbon molar ratio of the crude bio-oil.

[0055] The step of heating the wet biomass at the first temperature and the first pressure may also be conducted in an inert atmosphere, such as in the presence of at least one inert gas. Without intending to be bound by any particular theory, it is believed that heating the wet biomass in the presence of the catalyst in the inert atmosphere maximizes the crude bio-oil yield. The inert atmosphere may replace air inside of the reactor and minimize undesirable reactions that occur between the wet biomass and components in the air. The inert atmosphere may be present in the reaction vessel before the step of heating the wet biomass. One specific example of an inert gas is helium. However, other inert gases are also contemplated for use within the methods described herein. The inert atmosphere can be provided at a pressure ranging from 0 to 250 kPa, or, alternatively, from 50 to 225 kPa, or alternatively, from 70 to 200 kPa, or, alternatively, from 90 to 150 kPa. The inert atmosphere may be provided in the reaction vessel after evacuating at least a portion of the air from the reaction vessel. The evacuation of the air may be performed with devices that provide vacuum, e.g., the vacuum pump **26** as shown in FIG. **2**.

[0056] In one embodiment, the step of heating the wet biomass at the first temperature and the first pressure, for the time period ranging from 10 to 200 minutes, yields a crude bio-oil in an amount ranging from 20 to 80 wt. %, or, alternatively, from 30 to 70 wt. %, or, alternatively, from 40 to 60

wt. %, all based on the weight of the biomass solids before the step of heating at the first temperature and the first pressure.

[0057] It is believed that the crude bio-oil yield may exceed the lipid content of the biomass solids when cellular components other than triglycerides, such as protein, fiber, and carbohydrates of the biomass solids, are converted into crude bio-oil. The ability to convert additional cellular components into crude bio-oil may further broaden the scope of suitable sources of biomass solids.

[0058] Typically, the crude bio-oil comprises a liquid. In one embodiment, the crude bio-oil may include phenolic compounds, long-chain alkanes, and/or fatty acids. Specific examples of the fatty acids include, but are not limited to, palmitic acid and palmitoleic acid. Specific examples of long-chain alkanes include, but are not limited to, pentadecane, heptadecane, substituted hexadecanes, nonadecane, docosane, heptacosane, nonacosane, triacontane, hentriacontane, and heptacosane. The phenolic compounds may include alkyl phenols and benzenes. The crude bio-oil may also include other organic acids, long-chain hydrocarbons (unsaturated and saturated), indoles, piperidine derivatives, cholesterol, cholestane, cholestene, amides, other N-containing compounds, and combinations thereof.

[0059] The gas above the crude bio-oil may comprise CO_2, H_2, CH_4, and/or combinations thereof. In some embodiments, the gas above the crude bio-oil may also comprises N_2, C_2H_4, C_2H_6, and/or combinations thereof.

[0060] The crude bio-oil typically has an elemental composition of carbon in an amount ranging from 50 to 80 wt. %, hydrogen in an amount ranging from 1 to 20 wt. %, oxygen in an amount ranging from 5 to 20 wt. %, nitrogen in an amount ranging from 1 to 15 wt. %, and sulfur in an amount ranging from 0 to 5 wt. %, all based on the total weight of the crude bio-oil. Alternatively, the crude bio-oil has an elemental composition of carbon in an amount ranging from 60 to 80 wt. %, hydrogen in an amount ranging from 5 to 15 wt. %, oxygen in an amount ranging from 5 to 15 wt. %, nitrogen in an amount ranging from 1 to 10 wt. %, and sulfur in an amount of less than 0.01 to 3 wt. %, all based on the total weight of the crude bio-oil. Alternatively still, the crude bio-oil has an elemental composition of carbon in an amount ranging from 65 to 78 wt. %, hydrogen in an amount ranging from 8 to 12 wt. %, oxygen in an amount ranging from 6 to 12 wt. %, nitrogen in an amount ranging from 1 to 5 wt. %, and sulfur in an amount of less than 0.01 to 1 wt. %, all based on the total weight of the crude bio-oil.

[0061] The crude bio-oil may retain at least 50%, at least 60%, at least 70%, or at least 80% of carbon and hydrogen atoms originally present in the biomass solids. Typically, the crude bio-oil has a hydrogen to carbon molar ratio ranging from 1.0 to 2.0, or ranging from 1.5 to 2.0. The crude bio-oil also typically has an oxygen to carbon molar ratio ranging from 0.01 to 0.50, ranging from 0.01 to 0.20, or, alternatively, ranging from 0.06 to 0.10. In some embodiments, the crude bio-oil has a sulfur content below detection limits.

[0062] The crude bio-oil may have an overall aliphatic hydrogen content ranging from 60 to 99 wt. %, or, alternatively, from 70 to 99 wt. %, or, alternatively, from 80 to 99 wt. %, all based on the total amount of hydrogen in the crude bio-oil. Furthermore, the crude bio-oil may also have an alkyl carbon content ranging from 60 to 90 wt. % or, alternatively, from 70 to 99 wt. %, or, alternatively, from 80 to 99 wt. %, all based on the total amount of carbon in the crude bio-oil.

[0063] The crude bio-oil typically has a heating value ranging from 30 to 50 MJ/kg. Alternatively, the crude bio-oil may have a heating value ranging from 30 to 45 MJ/kg, or, alternatively, ranging from 35 to 45 MJ/kg.

[0064] After forming the crude bio-oil, the first catalyst may be removed from the crude bio-oil before further processing the crude bio-oil. In another embodiment, the first catalyst remains in the crude bio-oil and may be used in later steps.

[0065] After forming the crude bio-oil, the method also includes heating the crude bio-oil and the water at a second temperature and a second pressure for a time period of at least 30 minutes to form the upgraded bio-oil, with the proviso that at least a portion of the water remains in the liquid phase or is in the super-critical fluid phase throughout the step of heating to form the upgraded bio-oil, and with the proviso that the second temperature is greater than the first temperature and at least 300° C.

[0066] Without being limited to theory, heating the crude bio-oil and water at or near super-critical conditions of water converts the crude bio-oil into more upgraded bio-oil, having a higher concentration of fuel-grade components. By "super-critical conditions," it is intended to refer to a set of temperature and pressure conditions that are at or above the critical point of water, such that water becomes a super-critical fluid. The critical point of water is intended to mean the vapor-liquid critical point. The vapor-liquid critical point is where there are no longer phase boundaries between the liquid and gas phases. The vapor-liquid critical point comprises a critical temperature and a critical pressure. The critical temperature of water is about 374° C. and the critical pressure is about 22.064 MPa. Water at or near super-critical conditions exhibits properties that are very different from those of liquid water at room temperature. It has a lower dielectric constant, fewer and weaker hydrogen bonds, a higher native H^+ concentration (which facilitates acid-catalyzed reactions), and a higher solubility for small organic compounds. The heating of the crude bio-oil and water at or near super-critical conditions allows production of an upgraded bio-oil having higher heating values as compared to the crude bio-oil.

[0067] The step of heating the crude bio-oil and water may be conducted using techniques and devices known in the art. In one embodiment, the same reactor may be used for the step of heating the wet biomass and for the step of heating the crude bio-oil and water.

[0068] The crude bio-oil and water are typically included in a mass ratio ranging from 5:1 to 1:5, or, alternatively, from 2:1 to 1:2, or, alternatively, from 1:1, before the step of heating the crude bio-oil and water. Alternatively, it is also possible that no additional water is added to the reaction vessel in combination with the crude bio-oil.

[0069] The temperature at which the step of heating the crude bio-oil and water is referred to as the second temperature. Typically, the first second temperature is present in the reaction vessel throughout the step of heating the crude bio-oil and water to form the upgraded bio-oil. The second temperature is greater than the first temperature and is also at least 300° C. The second temperature may range from 300 to 650° C., or, alternatively, from 350 to 550° C., or, alternatively, from 375 to 425° C., or, alternatively, from 395 to 405° C. Other second temperatures are also contemplated, so long as at least a portion of the water present is in the liquid phase or is in a super-critical fluid phase throughout the step of heating the crude bio-oil and water to form the upgraded bio-oil.

[0070] As one of ordinary skill in the art will appreciate, by heating the crude bio-oil and water at the second temperature in the reactor, the pressure in the reaction vessel will naturally rise. The pressure may increase beyond what is provided by the presence of the reducing atmosphere or the inert atmosphere. The result of this pressure increase is predictable through means of one of ordinary skill in the art. In one possible configuration, the density within the reaction vessel can be monitored in order to indirectly ascertain the pressure within the reaction vessel throughout the step of heating the crude bio-oil at the second temperature.

[0071] During the step of heating the wet biomass at the second temperature, an elevated pressure may be present within the reaction vessel. The pressure present in the reaction vessel during the step of heating the water and crude bio-oil is referred to as the second pressure. Typically, the second pressure is present throughout the step of heating the water and crude bio-oil. The second pressure may range from 0.1 to 35 MPa, or, alternatively, from 10 to 30 MPa, or, alternatively, from 20 to 30 MPa.

[0072] In another embodiment, the second temperature and the second pressure are within super-critical conditions, such that at least a portion of the water is a super-critical fluid throughout the step of heating the crude bio-oil and water to form the upgraded bio-oil.

[0073] The crude bio-oil and water may be heated for a period of time of at least 30 minutes. Alternatively, the crude bio-oil and water may be heated for a time period ranging from 30 to 300 minutes, or, alternatively, from 120 to 250 minutes, or, alternatively, from 200 to 250 minutes. In one embodiment, the crude bio-oil and water may be heated for a time period of up to 480 minutes.

[0074] As set forth above, the first catalyst from the production of the crude bio-oil may remain in the crude bio-oil when the water and crude bio-oil undergo the step of heating at the second temperature and second pressure. Alternatively, the step of heating the crude bio-oil and water may be conducted in the presence of a second catalyst. In this embodiment, the second catalyst may be the same as the first catalyst described above, or may be different. Without intending to be bound by any particular theory, it is believed that using Pd/C, $Ni/SiO_2—Al_2O_3$, Ru/C, or zeolite as the second catalyst maximizes the mole fraction of H_2 produced in the upgraded bio-oil. Also, and without intending to be bound by any particular theory, it is believed that using Pd/C, Pt/C, Ru/C, or $Ni/SiO_2—Al_2O_3$ as the second catalyst maximizes the mole fraction of methane produced in the upgraded bio-oil. Without intending to be bound by any particular theory, it is believed that the $Ni/SiO_2—Al_2O_3$ catalyst has a desulfurization activity. Also, and without intending to be bound by any particular theory, it is believed that noble metal catalysts including, but not limited to, Pd/C, Pt/C, Ru/C maximize the hydrogen to carbon molar ratio of the upgraded bio-oil.

[0075] It is also contemplated that the step of heating at the second temperature and the second pressure may be conducted in the absence of the first and the second catalyst. Without intending to be bound by theory, the fatty acid content appears the highest in the upgraded bio-oil formed when heating the crude bio-oil and water at the second temperature and the second pressure in the absence of the first and second catalyst. The resulting upgraded bio-oil also has a high viscosity compared with those produced under catalytic conditions.

[0076] If used, the second catalyst may be included with the crude bio-oil and water in an amount ranging from 10 to 50 wt. %, or, alternatively, from 10 to 40 wt. %, or, alternatively, from 20 to 30 wt. %, all based on the total weight of the crude bio-oil. The second catalyst may comprise Pd/C, Pt/C, Ru/C, $Ni/SiO_2—Al_2O_3$, sulfided $CoMo/y\text{-}Al_2O_3$, zeolite, activated carbon, or combinations thereof.

[0077] In another embodiment, the step of heating the crude bio-oil and the water at the second temperature and the second pressure may be conducted in the presence of the reducing atmosphere. It is believed that heating the crude bio-oil in the reducing atmosphere yields reduced amounts of CO_2 in the gas above the upgraded bio-oil and minimizes production of C_2H_4, which results in a higher quality upgraded bio-oil. It is also theorized that the heating the crude bio-oil and water in a reducing atmosphere removes even more of the original nitrogen atoms present in the biomass solids. In the presence of a reducing atmosphere, the crude bio-oil yield and heating value are largely insensitive to the presence or identity of the catalyst. In one embodiment, the reducing atmosphere comprises pressurized hydrogen gas. Without being bound to any particular theory, it is believed that the pressurized hydrogen or other reducing agent may hydrogenate the unsaturated heterocyclic rings and crack subsequently produced products. However, it is also contemplated that the reducing atmosphere may comprise other gaseous reducing agents.

[0078] The reducing atmosphere may be provided at a pressure ranging from 0.1 to 30 MPa. In other embodiments, the reducing atmosphere may be provided at a pressure ranging from 20 to 30 MPa, or, alternatively, from 5 to 25 MPa.

[0079] The step of heating the crude bio-oil and water to form the upgraded bio-oil may also be conducted in an inert atmosphere, such as in the presence of at least one inert gas. Without intending to be bound by any particular theory, it is believed that heating the crude bio-oil and water in the inert atmosphere maximizes the yield of the upgraded bio-oil. The inert atmosphere may replace air inside of the reactor and minimize undesirable reactions that occur between the crude bio-oil and components in the air. One specific example of an inert gas is helium. However, other inert gases are also contemplated for use with the methods described herein. The inert atmosphere may be provided the reaction vessel after evacuating at least a portion of the air from the reaction vessel.

[0080] In still another embodiment, the step of heating the crude bio-oil and water to form the upgraded bio-oil may also be conducted under acidic or basic conditions. For example, in one embodiment, HCl or NaOH may be added to the water and crude bio-oil to adjust the pH of the solution before or throughout the step of heating the crude bio-oil and water to form the upgraded bio-oil.

[0081] Typically, the upgraded bio-oil comprises liquid. Typically, the upgraded bio-oil comprises hydrocarbons. In one embodiment, the upgraded bio-oil comprises alkanes and aromatics. Specific examples of alkanes include, but are not limited to, pentadecane, other alkanes ranging from C_9 to C_{33}, and combinations thereof. The aromatics may include, but are not limited to, alkylphenols and benzenes.

[0082] The gas above the upgraded bio-oil may comprise CO_2, H_2, CH_4, C_2H_6, and combinations thereof. Typically, the gas above the upgraded bio-oil comprises a total amount of gas ranging from 1.0 to 4.0 wt. %, or, alternatively, from 1.5 to 3.0 wt. %, both based on the total weight of the upgraded bio-oil.

[0083] In one specific embodiment, the proportion of saturated hydrocarbons and aromatic compounds in the upgraded bio-oil increased about 500% compared to the crude bio-oil. A likely source of the saturated compounds is decarboxylation or deoxygenation of the fatty acids.

[0084] In one or more embodiments, the upgraded bio-oil comprises more low-boiling species than the upgraded bio-oil. This physical change is also apparent in the upgraded bio-oil being a freely flowing liquid whereas the crude bio-oil is more of a tarry consistency.

[0085] Without being limited by theory, it is believed that the step of heating the crude bio-oil and water at the second temperature and the second pressure eliminates some of the fatty acids present in the crude bio-oil and reduced the relative amount of others. In one specific embodiment, the area % for palmitic acid decreased from 14.2% in the crude bio-oil to 2.9% in the upgraded bio-oil (Pt/C, H_2, NaOH). At the same time, the relative amount of pentadecane in the upgraded bio-oil is much higher than that in the crude bio-oil. A likely pathway for the formation of pentadecane is the decarboxylation of palmitic acid, catalyzed by Pt/C. Palmitoleic acid and its derivatives are the second most abundant fatty acids in the crude bio-oil (6.8 area %). After upgrading, no palmitoleic acid and none of its derivatives were detected. This result indicates a hydrogenation and/or decarboxylation pathway. Similar decarboxylation pathways are likely operative for the other fatty acids in the crude bio-oil.

[0086] The relative amount of fatty acid amides (myristamide, palmitamide, and stearamide) and nitriles increases after the step of heating the crude bio-oil and water at the second temperature and the second pressure. A possible pathway for the formation of amides and nitriles in the step of heating the crude bio-oil and water at the second temperature and the second pressure is the reaction of fatty acids with ammonia.

[0087] The carbon and hydrogen content of the upgraded bio-oil is typically higher than that of the crude bio-oils, whereas the oxygen and nitrogen content in the upgraded bio-oil is also typically lower than the crude bio-oil. Therefore, the increased carbon and hydrogen levels and reduced oxygen content lead to the upgraded bio-oil having a higher energy density than the crude bio-oil. No sulfur was detected in any of the upgraded bio-oils. Furthermore, upgraded bio-oils formed in a reducing atmosphere, even in the absence of catalyst, may exhibit several desirable changes as compared to the crude bio-oil. More specifically, these upgraded bio-oils have higher carbon and hydrogen content and lower sulfur, oxygen, and nitrogen content than the crude bio-oil.

[0088] The hydrogen to carbon ratio is about the same in both the crude bio-oil and the upgraded bio-oil that is formed in the presence of the second catalyst at the second temperature and the second pressure, but the oxygen to carbon atomic ratio drops and the nitrogen to carbon atomic ratio drops. These findings indicate that deoxygenation or hydrodeoxygenation proceeded during the step of heating the crude bio-oil and water at the second temperature and the second pressure. Upgraded bio-oil typically has a lower proportion of nitrogen than the crude bio-oil. The heating of the crude bio-oil and water at the second temperature and the second pressure shows activity for sulfur removal from the crude bio-oil.

[0089] Under the experimental conditions tested, presence of the Pt/C catalyst and a reducing atmosphere led to the upgraded bio-oil having the lowest total acid number (25.3), indicating that most of the fatty acids in the crude bio-oil were removed.

[0090] The upgraded bio-oil typically has an elemental composition of carbon in an amount ranging from 70 to 90 wt. %, hydrogen in an amount ranging from 1 to 20 wt. %, oxygen in an amount ranging from 1 to 15 wt. %, nitrogen in an amount ranging from 1 to 15 wt. %, and sulfur in an amount less than 1 wt. %, all based on the total weight of the upgraded bio-oil. Alternatively, the upgraded bio-oil has an elemental composition of carbon in an amount ranging from 75 to 85 wt. %, hydrogen in an amount ranging from 5 to 15 wt. %, oxygen in an amount ranging from 1 to 10 wt. %, nitrogen in an amount ranging from 1 to 10 wt. %, and sulfur in an amount less than 0.10 wt. %, all based on the total weight of the upgraded bio-oil. Alternatively still, the upgraded bio-oil has an elemental composition of carbon in an amount ranging from 80% to 85% by weight, hydrogen in an amount ranging from 10% to 15% by weight, oxygen in an amount ranging from 3% to 6% by weight, nitrogen in an amount ranging from 2% to 3% by weight, and sulfur in an amount of less than 0.01% by weight, all based on the total weight of the upgraded bio-oil.

[0091] The upgraded bio-oil may include at least 60%, alternatively, at least 70%, or alternatively, at least 80% of carbon atoms originally present in the crude bio-oil and the gas above the crude bio-oil. The upgraded bio-oil may also include at least 50%, alternatively at least 60%, or alternatively, at least 70% of hydrogen atoms originally present in the crude bio-oil and the gas above the crude bio-oil.

[0092] The upgraded bio-oil may have a hydrogen to carbon molar ratio ranging from 1.0 to 2.0, or, alternatively, from 1.5 to 2.0. The upgraded bio-oil may also have an oxygen to carbon molar ratio ranging from 0.01 to 0.10, or, alternatively, from 0.01 to 0.05, or, alternatively, from 0.03 to 0.05. Additionally, the upgraded bio-oil may have a nitrogen to carbon molar ratio ranging from 0.01 to 0.10, or, alternatively, from 0.01 to 0.05.

[0093] The upgraded bio-oil may have a total acid number (TAN) ranging from 10 to 80 mg KOH/g, or alternatively, ranging from 20 to 75 mg KOH/g. The TAN is a common quality measurement for liquid fuels. The TAN indicates whether a given fuel may be corrosive. Bio-oil having a low TAN value are considered "safer" for storage and transportation, whereas bio-oil having higher TAN values are considered more prone to lead to operational problems and cause corrosion during storage.

[0094] The upgraded bio-oil typically has a heating value ranging from 35 to 55 MJ/kg. Alternatively, the upgraded bio-oil may have a heating value ranging from 40 to 50 MJ/kg, or ranging from 40 to 45 MJ/kg.

EXAMPLES

[0095] The Examples defined in this section are merely examples of the many possible configurations for the present invention. More particularly, the exemplary reactor types and sizes, along with the amounts of the various reactants, catalysts, and auxiliary components, are contemplated to vary depending on the industrial setting for which they are utilized. Accordingly, the wet biomass can be processed in any amount desired, and the other components and size and design of reactors can be modified accordingly.

[0096] 316-stainless steel reactors (herein after "crude bio-oil reactors") are used, which allow for the recovery and

analysis of both the crude bio-oil and the gas above the crude bio-oil in a single run. The crude bio-oil reactors, illustrated in FIG. **1**, may comprise an 8-in length of ¾-in O.D. tube having a first end opposite and spaced from a second end in addition to a wall thickness of 0.065 inches. Additionally, the crude bio-oil reactors have a volume of 31 ml. A cap is placed on the first end of the O.D. tube, and a cap assembly comprising an 8.8-in length of ¼-inches O.D. tube having a wall thickness of 0.035 inches is fitted on the second end. The cap assembly is connected to and in fluid communication with a HiP high-pressure valve.

Procedure

[0097] Prior to heating the wet biomass at form the crude bio-oil at the first pressure and the first temperature, the crude bio-oil reactor is loaded with water and seasoned at 350° C. for 60 minutes to remove any residual organic material from the crude bio-oil reactor and to expose the fresh metal walls to high temperature water. Then, the crude bio-oil reactor is gradually cooled to ambient temperature, thoroughly washed with acetone, and air-dried. Once air-dried, the crude bio-oil reactor is pressure tested with helium.

[0098] In a typical experiment, 0.384 g of the first catalyst is loaded into the crude bio-oil reactor. This amount represents a 50 wt. % loading of the first catalyst with respect to microalgae paste (biomass solids) on an ash-free basis. Once the catalyst is loaded into the crude bio-oil reactor, 4.27 g of microalgae paste is loaded, prior to adding 13.5 ml of freshly deionized water. This represents "wet biomass." The amount of water is selected such that 95% of the crude bio-oil reactor volume is occupied by liquid during the heating step at the first temperature and the first pressure, if water were the sole component in the crude bio-oil reactor. Once the crude bio-oil reactor is loaded, the cap assembly is connected and securely tightened to seal the crude bio-oil reactor. Air inside the crude bio-oil reactor is displaced with the inert atmosphere comprising helium following repeated cycles of evacuation and charging with helium (70 kPa, gauge). The 70 kPa of helium also serves as an internal standard for the quantification of yield of the gas above the crude bio-oil. In some experiments, the crude bio-oil reactor is further charged with the reducing atmosphere comprising hydrogen at a pressure of 3500 kPa. After charging of helium, and in some instances hydrogen, a high-pressure valve is securely tightened and the crude bio-oil reactor is disconnected from any source of gas.

[0099] The crude bio-oil reactors are placed vertically in a Techne Fluidized Sand Bath (model SBL-2), and the crude bio-oil reactors are maintained at one exemplary first temperature of 350° C. by a Techne TC-8D temperature controller with a precision of ±2° C. Heat up time of the crude bio-oil reactors at the first temperature is about 3 minutes and step of heating at the first temperature and the first pressure has a duration of 60 minutes.

[0100] For analysis purposes, the crude bio-oil reactors are removed from the sand bath and immersed in a cold-water bath for about 5 minutes to quench the reaction and cease the heating step. The crude bio-oil reactors are further cooled in a refrigerator for 30 minutes. The crude bio-oil reactors are then removed from the refrigerator and placed in ambient conditions for at least 15 hours to allow the crude bio-oil and the gas above the crude bio-oil to equilibrate.

[0101] The analysis of the gas above the crude bio-oil is performed with an Agilent Technologies model 6890N gas chromatograph (GC) equipped with a thermal conductivity detector (TCD). A 15-ft×⅛-inches i.d. stainless steel column, packed with 60×80 mesh Carboxen 1000 (Supleco) separated each component in the gas above the crude bio-oil. Argon (15 ml min^{-1}) serves as a carrier gas for the analysis.

[0102] The crude bio-oil reactor is connected to a GC gas sampling valve, and the gas above of the crude bio-oil in the crude bio-oil reactors flows into a sample loop as the reactor valve is opened slowly and slightly to allow about a 1 mL sample to exit. The sample is sent to a column via a Valco switching valve, which is automated with an air actuator. After the switching valve closes, the reactor valve is also closed. To ensure that the GC sample is representative of the gas above the crude bio-oil in the crude bio-oil reactor, a subsequent analysis is conducted. Thus, two consecutive analyses of the gas above the crude bio-oil are taken for each crude bio-oil reactor. Initially, the column is held at a temperature of 35° C. for 5 minutes and then increased to a final temperature of 225° C. at a rate of 20° C. min^{-1}. The final temperature of the column is held for 15 minutes.

[0103] A mole fraction of each constituent in the gas above the crude bio-oil is determined via calibration curves generated from analysis of the analytical gas standards with known compositions. The amount of helium added to the crude bio-oil reactor is used as an internal standard to determine the molar amount of each constituent present in the gas above the crude bio-oil. The yield of each constituent is calculated by dividing the molar amount of the constituent by mass of the biomass solid loaded into the crude bio-oil reactor.

[0104] After the gas above the crude bio-oil has been analyzed, the crude bio-oil reactors are opened to recover the crude bio-oil in each crude bio-oil reactor. Approximately 10 mL of dichloromethane is added to the crude bio-oil, after which the crude bio-oil reactor is closed, (i.e., capped) and shaken vigorously by hand. Contents of the crude bio-oil reactor, including the first catalyst, are transferred to a glass separatory funnel. The crude bio-oil reactor is washed twice more with an additional 10 mL aliquot of dichloromethane to ensure that the crude bio-oil is completely collected and transferred to the separatory funnel. Once the crude bio-oil is transferred to the separatory funnel, the separatory funnel is sealed and shaken to mix the contents. The contents separate into an organic phase and an aqueous phase.

[0105] The organic phase, containing the first catalyst, is withdrawn and retained for catalyst separation. The aqueous phase is extracted with sequential 20 mL and 10 mL aliquots of dichloromethane. All of these dichloromethane extracts are combined and any first catalyst present is recovered by filtering the combined dichloromethane extracts. The filtrate of the combined dichloromethane extracts is transferred to a round-bottom flask, and the dichloromethane is evaporated under a vacuum of 27 kPa at 30° C. for 5 minutes. The round-bottom flask is quickly capped and weighed with any remaining material to determine the mass of the remaining material. The material remaining in the round-bottom flask is the crude bio-oil and residual dichloromethane. To estimate an amount of the residual dichloromethane remaining, a control experiment is conducted wherein a flask containing only dichloromethane is subjected to evaporation as described above. The mass of residual dichloromethane (0.17 g) in the control experiment is subtracted from the mass of the remaining material to estimate the mass of the crude bio-oil produced in each experiment. The crude bio-oil yield is determined by dividing the mass of the crude bio-oil by the mass of dry microalgae loaded into the reactor. Since the amount of residual solvent in each sample is estimated as described above, the crude bio-oil yields reported are necessarily approximate and subject to systematic error. Nevertheless, the trends in the crude bio-oil yields, which should be largely insensitive to any systematic error, are more reliable.

[0106] An Agilent Technologies 6890N GC equipped with an auto-sampler, an auto- injector, and a mass spectrometric detector is used to analyze the gas above the crude bio-oil (redissolved in dichloromethane). An Agilent J&W DB-5HT non-polar capillary column (30 m length, 0.250 mm i.d., 0.10 μm film thickness) is used to separate the different constituents of the crude bio-oil. A sample of the crude bio-oil having a volume of 2 μl is injected, and an inlet split ratio was 5:1. The non-polar capillary column was held at an initial temperature of 80° C. for 2 minutes. The temperature of the non-polar capillary column is then ramped to 300° C. at 3° C. min^{-1} and held isothermally for 10 minutes, having a total runtime of about 85 minutes. Helium serves as a carrier gas (20 mL min^{-1}). A Wiley mass spectral library is used for constituent identification.

[0107] ^{1}H NMR and ^{13}C NMR spectra of the gas above the crude bio-oil are recorded at 400.0 MHz and 100.6 MHz, respectively, on a 400-MHz NMR spectrometer (Inova 400, Varian) at a temperature of 25° C. The crude bio-oil (8 wt. %) is dissolved in $CDCl_3$. About 10^5 scans are accumulated for the ^{13}C spectrum using a 45° pulse width together with broad band proton decoupling. NMR tubes of 5 mm diameter are used.

[0108] Fourier transform infrared spectroscopic analysis (FT-IR) is performed on a Nicolet 6700 FT-IR Spectrometer (Thermo Electron Corporation) to determine the functional groups of the crude bio-oil. FT-IR spectra (resolution: 4 cm^{-1}, scan: 254, range: 4,000-400 cm^{-1}) are taken after spreading a thin film of the crude bio-oil on a multi-bounce plate of ZnSe crystal at a controlled ambient temperature (25° C.). A background spectrum is also collected under identical conditions.

[0109] An elemental composition (carbon, hydrogen, oxygen, nitrogen, and sulfur) of the biomass solids (i.e., dry microalgae) and the crude bio-oil are determined by Atlantic Microlab, Inc. All residual dichloromethane is evaporated prior to elemental analysis. The higher heating values of the crude bio-oil and the dry microalgae are estimated by using the Dulong formula provided below:

$$\text{Heating Value (MJ/kg)} = 0.338C + 1.428(H - O/8) + 0.095S$$

wherein C, H, O, and S are the wt. % composition of each element in the organic material.

[0110] The gas above the crude bio-oil typically comprises H_2, CO_2, and CH_4, with lesser amounts of C_2H_4 and C_2H_6. Typical hydrogen to carbon and oxygen to carbon molar ratios for the crude bio-oil are 1.7 and 0.09, respectively.

[0111] FIG. **3** shows the effects of various catalysts on the crude bio-oil yields when present during the heating of the wet biomass at the first temperature and the first pressure in both the reducing atmosphere and an inert atmosphere. The crude bio-oil yields range from a low of 35% from an uncatalyzed heating of the wet biomass in an inert atmosphere at the first temperature and the first pressure to a high of 57% from a Pd/C catalyzed heating of the wet biomass in an inert atmosphere at the first temperature and the first pressure.

[0112] All of the crude bio-oil lipid contents exceed the crude lipid content (28 wt. %) of the biomass solids. This result demonstrates that cellular components, e.g., protein, fiber, and carbohydrate, in addition to triglycerides present in the biomass solids, are being converted into the crude bio-oil.

[0113] Addition of first catalysts promoted production of crude bio-oil during heating at the first temperature and the first pressure in the inert atmosphere. With the exception of the high yield from Pd/C, the crude bio-oil yields from all other first catalysts in the inert atmosphere are similar (about 45-50%), but higher than the crude bio-oil yield from heating in the absence of a catalyst. The crude bio-oil yield from heating at the first temperature and the first pressure in the presence of the zeolite first catalyst show the greatest variability. This variability is likely related to difficulty in recovering all of the crude bio-oil in these experiments because the crude bio-oil has a high viscosity when zeolite is used as the first catalyst.

[0114] FIG. **3** also shows that heating in the reducing atmosphere at the first temperature and the first pressure resulted in a higher crude bio-oil yield without any catalysts. The crude bio-oil yields from heating at the first temperature and the first pressure in the reducing atmosphere were similar (given standard deviations), regardless of the presence or absence of a catalyst or catalyst identity. If the first catalyst promoted hydrocracking reactions, wherein large molecules are converted to smaller, more volatile ones, the crude bio-oil yield would be reduced because more of these smaller, and lighter, molecules would be lost during the solvent evaporation stage of bio-oil recovery.

[0115] Tables 3a and 3b provide elemental compositions and estimated higher heating values of upgraded bio-oil formed from the step of heating the crude bio-oil and water at the second temperature and the second pressure (described in greater detail above in the Detailed Description). The crude bio-oil has a carbon and hydrogen content greater than that of the biomass solids. However, the crude bio-oil has an oxygen content (8.3-9.6 wt. %) less than that of the biomass solids (26 wt %). Increased carbon and hydrogen levels in combination with reduced oxygen content results in the crude bio-oil having a higher energy density than the biomass solids. The crude bio-oil has heating values ranging from about 38 to about 40 MJ/kg, which exceeds heating values of other bio-oils produced from pyrolysis of terrestrial wet biomass. Additionally, the heating values of the crude bio-oil are close to heating values of petroleum-derived fuels (about 42 MJ/kg). The oxygen content of the crude bio-oil is also lower than that of other bio-oils produced from pyrolysis of terrestrial wet biomass.

TABLE 3a

Elemental Composition and Heating Value of Crude Bio-oil (Inert Atmosphere)

	Dry Algae	No Cat.	Pd/C	Pt/C	Ru/C	$Ni/SiO_2—Al_2O_3$	CoMo/γ-Al_2O_3	Zeolite
C (wt. %)	43.0	75.3	73.4	75.9	72.6	75.0	75.7	69.6
H (wt. %)	5.97	10.2	10.8	10.8	10.3	10.2	10.3	9.44
O (wt. %)	25.8	9.18	9.01	8.48	9.34	8.59	8.50	9.46
N (wt. %)	6.32	4.18	3.88	4.04	3.49	3.74	4.31	4.33

TABLE 3a-continued

Elemental Composition and Heating Value of Crude Bio-oil (Inert Atmosphere)

	Dry Algae	No Cat.	Pd/C	Pt/C	Ru/C	$Ni/SiO_2—Al_2O_3$	CoMo/γ-Al_2O_3	Zeolite
S (wt. %)	0.58	0.84	0.52	0.72	0.31	—	0.58	0.74
H/C	1.67	1.63	1.76	1.71	1.70	1.62	1.63	1.63
O/C	0.45	0.091	0.092	0.084	0.097	0.086	0.084	0.100
HHV (MJ/kg)	18.5	38.5	38.6	39.6	37.5	38.3	38.8	35.4

TABLE 3b

Elemental Composition and Heating Value of Crude Bio-oil (Reducing Atmosphere - 3500 kPa Reducing Atmosphere)

	Dry Algae	No Cat.	Pd/C	Pt/C	Ru/C	$Ni/SiO_2Al_2O_3$	CoMo/γ-Al_2O_3	Zeolite
C (wt. %)	43.0	75.5	74.9	76.1	73.2	76.2	74.8	74.2
H (wt. %)	5.97	10.5	10.6	11.1	10.6	10.7	10.4	10.5
O (wt. %)	25.8	9.23	9.04	8.34	8.63	9.01	8.55	8.92
N (wt. %)	6.32	4.08	4.20	3.92	3.33	3.64	3.99	4.05
S (wt. %)	0.58	0.69	0.65	0.62	0.42	—	0.56	0.88
H/C	1.67	1.68	1.70	1.74	1.74	1.68	1.66	1.69
O/C	0.45	0.092	0.090	0.082	0.088	0.089	0.086	0.090
HHV (MJ/kg)	18.5	39.0	38.9	40.1	38.4	39.4	38.6	38.5

[0116] Reactions without catalysts present form a crude bio-oil having a higher heating value and lower oxygen content than that of the biomass solids. Reactions with supported noble metal catalysts such as Pt/C, Pd/C, and Ru/C present and conducted in an inert atmosphere increase the hydrogen to carbon molar ratios of the crude bio-oil formed therefrom. The presence of Pt, $Ni/SiO_2Al_2O_3$, and CoMo/γ-Al_2O_3 catalysts lead to crude bio-oil having decreased oxygen to carbon ratios as compared to crude bio-oil formed in the absence of these catalysts. Findings indicate that catalytic deoxygenation or hydrodeoxygenation proceeded during reactions.

[0117] Except when Pd/C was present, heating performed in the presence of the reducing atmosphere resulted in crude bio-oil having an increased hydrogen content and an increased hydrogen to carbon molar ratio compared to crude bio-oil formed from heating in inert atmospheres at otherwise identical conditions. Heating performed in the presence of the reducing atmosphere also formed crude bio-oil having a higher heating value as compared to crude bio-oil formed from heating in inert atmospheres at otherwise identical conditions.

[0118] The biomass solids usually contain a high proportion of nitrogen due to the presence of chlorophyll and protein. The biomass solids used in the experiments above have a nitrogen content of 6.3 wt. % on a dry basis. However, the crude bio-oil formed therefrom has a lower proportion of nitrogen than the biomass solids. Of the first catalysts tested, Ru/C and Ni/SiO_2-Al_2O_3 produced crude bio-oils with the lowest nitrogen wt %, both in the inert atmosphere and the reducing atmosphere. The reducing atmosphere led to a lower nitrogen wt % for all crude bio-oils except when the Pd/C catalyst is present during reaction. Although Ru/C and CoMo/γ-Al_2O_3 first catalysts exhibit some activity for sulfur removal from the resulting crude bio-oil, crude bio-oil formed in the presence of $Ni/SiO_2—Al_2O_3$ contained no detectable sulfur. Seemingly, $Ni/SiO_2—Al_2O_3$ provides complete desulfurization of the crude bio-oil.

[0119] GC-MS is used to separate and identify molecular components in the crude bio-oil from Pd/C-catalyzed heating at the first temperature and the first pressure in the inert atmosphere. FIG. **4** provides a representative total ion chromatogram for the crude bio-oil, dissolved in dichloromethane, containing more than 100 peaks. A mass spectral library and computer matching are used to facilitate compound identification. FIG. **4** provides tentative identities of any major (peak area at least 0.5% of the total) individual molecular components in the crude bio-oil.

[0120] The crude bio-oil comprises fatty acids (peaks 9, 14, 15, 16, and 19), phenolic compounds (peaks 1, 3), and different long-chain alkanes. The fatty acids (over ⅓ of the total peak area) comprise at least palmitic acid and palmitoleic acid. These fatty acids may arise from hydrolysis of triacylglycerols present in the biomass solids. The different long-chain alkanes (about 20% of the total peak area) comprise at least pentadecane, heptadecane, substituted hexadecanes, nonadecane, docosane, heptacosane, nonacosane, triacontane, hentriacontane, and heptacosane.

[0121] FIG. **5** provides three total ion chromatograms of crude bio-oils formed form heating at the first temperature and the first pressure without a catalyst present, with Pd/C catalyst present in an inert atmosphere, and with Pd/C catalyst present in a reducing atmosphere. Peak regions labeled "a" and "b" represent fatty acids and alkanes, respectively. As is evident from FIG. **5**, non-catalytic heating produced a crude bio-oil having more fatty acids than alkanes. Heating in the presence of the Pd/C catalyst and an inert atmosphere forms crude bio-oil having peak heights in both the alkane and the fatty acid regions (center chromatogram) which are comparable to crude bio-oils formed from non-catalytic reactions. Heating in the presence of the Pd/C catalyst and a reducing atmosphere forms crude bio-oil having alkane peak heights exceeding those of the fatty acid region (top chromatogram). These results are consistent with the Pd/C catalyst being

effective for deoxygenating fatty acids. Converting fatty acids to alkanes improves the storage stability of the crude bio-oil and also maximizes energy density of the crude bio-oil.

[0122] ^{1}H and ^{13}C NMR spectroscopies identify the types and amounts of functional groups in the crude bio-oil. The chemical shift provides information about the identity of the functional group, and the peak areas provide information about relative abundance of the functional group. NMR analysis was conducted on the crude bio-oil obtained from heating at the first temperature and the first pressure in the presence of the Pd/C catalyst and an inert atmosphere.

[0123] FIG. **6** shows the ^{1}H NMR spectrum. Table 4 identifies functional groups associated with different ranges of chemical shifts and provides relative abundance of each functional group as determined from the integrated peak areas in FIG. **6**. Most of the strong peaks reside within the 0.0-2.2 ppm range, where aliphatic methyl and methylene protons appear, along with aliphatic hydroxyls. Integrating these peaks leads to an estimate of the overall aliphatic hydrogen content as 80% in the crude bio-oil. This high content of aliphatic protons is consistent with the molecular identities shown in FIG. **4** for the major peaks in the total ion chromatogram. Protons in $CH_3C(=O)—$, $CH_3—Ar$, and $—CH_2Ar—$ functionalities appear in the 2.2-3.0 ppm range, and the peak area in this range gives a hydrogen content of 11%. Aromatic and carboxylic acid protons appear around 8-10 ppm, and the integrated peak area in this region was 1.4%, consistent with the identification of fatty acids and phenols in the GC-MS analysis.

TABLE 4

NMR Chemical Shift Assignments and Relative Abundances for Crude Bio-oil (Pd/C, Inert Atmosphere)

Chemical Shift region (ppm)	Type of Protons	Hydrogen Content (% area of total)
10.0-8.0	—CHO, —COOH, downfield ArH	1.4
8.0-6.8	ArH, HC=C (conjugated)	4.0
6.8-6.4	HC=C (nonconjugated)	0.0
6.4-4.2	$—CH_n—O—$, ArOH, HC=C (nonconjugated)	2.9
4.2-3.0	$CH_3O—$, $—CH_2O—$, —CHO—	1.2
3.0-2.2	$CH_3C(=O)—$, $CH_3—Ar$, $—CH_2Ar—$	11.3
2.2-1.6	$—CH_2—$, aliphatic OH	7.3
1.6-0.0	$—CH_3$, $—CH_2—$	71.8

[0124] FIG. **7** provides the ^{13}C NMR spectrum of the crude bio-oil, and Table 5 provides functional group analysis and integrated areas in specific chemical shift ranges. The ^{13}C NMR spectrum was divided into five chemical shift ranges for analysis: 214-160 ppm (carbonyl carbons), 160-110 ppm (aromatic carbons), 110-84 ppm (carbohydrate), 84-64 ppm (methoxy- or hydroxyl-bound carbons), and 64-1 ppm (alkyl carbons). The alkyl region was also subdivided into 33-23 ppm (mostly secondary and tertiary carbons) and 23-6 ppm (mostly primary and secondary carbons). The crude bio-oil had high alkyl carbon content (83%), which is consistent with the high content of aliphatic hydrogen observed in the ^{1}H NMR spectrum and the abundant presence of alkanes and fatty acids in the total ion chromatogram. Weak resonance from carbohydrate carbons was detected in the crude bio-oil. It is worth noting that the crude bio-oil was derived from wet biomass comprising 12 wt. % of carbohydrate. This finding suggests that the carbohydrate of the biomass solids was converted to other products in the crude bio-oil. The crude bio-oil has a total aromatic carbon content is 14.9%.

[0125] On the basis of the GC-MS analysis, most of the aromatic compounds are derivatives of alkylphenols and benzenes. The presence of carbonyl carbon atoms, as well as the proportions present (~1.4%), is consistent with the high fatty acid content in the crude bio-oil revealed by the GC-MS analysis. Moreover, the proportion present (~1.4%) is also consistent. GC-MS analysis indicated that the crude bio-oil is more than ⅓ fatty acid, and that the C_{16} fatty acids dominate. Thus, a rough estimate for the proportion of carbonyl carbon would be $⅓×1/16$, or about 2%, a value not too different from the experimental value of 1.4%.

TABLE 5

^{13}C NMR Peak Assignments and Integration Results for Crude Bio-oil (Pd/C, Inert Atmosphere)

Chemical Shift (ppm)	Type of Carbons	Carbon Content (% area of total)
214-160	carbonyl	1.4
160-110	aromatic	14.9
110-84	carbohydrate	0.6
84-64	methoxy/hydroxy	0.1
64-1	alkyl carbon	82.8
33-23	secondary and tertiary alkyl carbons	46.4
23-6	primary and secondary alkyl carbons	15.3

FT-IR Analysis

[0126] FIG. **8** is a FT-IR spectrum of the crude bio-oil. The GC-MS and NMR results indicated that the crude bio-oil comprises primarily methylene groups either in alkanes or fatty acids. The IR results are fully consistent with this characterization. The C—H stretching vibration between 2850 and 2900 cm^{-1} and the C—H deformation vibrations between 1375 and 1475 cm^{-1} indicate the presence of aliphatic hydrogen atoms. The high intensity in these two regions suggests that a significant amount of the hydrogen in the crude bio-oil is aliphatic. Coupled C—O stretch vibrations in COOH groups appear between 1300 and 1420 cm^{-1}, and the presence of a peak in this region is consistent with the presence of fatty acids in the crude bio-oil. In addition, the absorbance between 1640 and 1715 cm^{-1} represents C=C and C—N (amide carbonyl) stretching vibrations which are indicative of alkenes and amides.

Effect of Catalysts on Gas Yields and Composition

[0127] FIG. **9** illustrates effects of different catalysts on the composition of the gas above the crude bio-oil. The step of heating the crude bio-oil and water at the second temperature and the second pressure was conducted in an inert atmosphere. The gas above the crude bio-oil typically comprises CO_2, H_2, and CH_4. CO_2 may be formed from reactions such as steam reforming and water gas shift, which also produce hydrogen.

Steam reforming $C_xH_yO_z+(2x-z)H_2O \rightarrow xCO_2+(y/2+2x-z)H_2$

Water-gas shift reaction $CO+H_2O \leftrightarrow CO_2+H_2$

[0128] The H_2 is present in smaller amounts than CO_2, therefore indicating that much of the CO_2 must form from other reactions. The existence of separate pathways for CO_2

and H_2 formation is also supported by the disparity in CO_2 and H_2 activation energies for formation from wet biomass (38±3 kJ/mol vs. 99±5 kJ/mol, respectively) during uncatalyzed heating. Methane may form via a methanation reaction.

$$CO+3H_2 \leftrightarrow CH_4+H_2O$$

This route is likely, especially for the runs with the Ru/C and Ni/SiO_2—Al_2O_3 catalysts. Both the Ru/C and Ni/SiO_2—Al_2O_3 catalysts are known to have methanation activity. CO was not detected in the gas above the crude bio-oil, possibly because any CO that formed would have been consumed in the water gas shift and methanation reactions by the end of the 60-minute experiment.

[0129] It is clear from FIG. **9** that the presence and type of catalyst affects the gas composition. Pd/C, Ni/SiO_2—Al_2O_3, and zeolite increase the mole fraction of hydrogen relative to uncatalyzed reactions. Pd/C, Pt/C, Ru/C, and Ni/SiO_2—Al_2O_3 all increase the mole fraction of methane, although Ru/C and Ni/SiO_2—Al_2O_3 exhibit the greatest effect. Methane was the most abundant component in the gas above the crude bio-oil from Ru/C catalyzed reactions. The N_2 mole fraction was appreciable only when the zeolite catalyst was present during reactions. The source of the N_2 may be ammonia, which can form from hydrolysis of amino acids that may be produced by hydrothermal decomposition of proteins in the biomass solids. The biomass solids used herein contain greater than 50 wt % protein. C_2H_4, C_2H_6 and N_2 were also produced in the gas above the crude bio-oil but in small amounts that varied with the type of catalyst present during reactions.

[0130] Results from the heating done in a reducing atmosphere appear in FIG. **10**. The mole fractions shown are calculated on a H_2-free basis, therefore the mole fractions reflect relative amounts of the different gases that are solely products of heating the wet biomass in the presence of the catalyst. H_2 is also a potential product; however, H_2 may be present at the start of the reaction so any H_2 would also be a potential reactant. The trends with respect to methane and nitrogen formation are the same as those for the runs in the inert atmosphere. Namely, the Ru/C and Ni/SiO_2—Al_2O_3 catalysts lead to high methane mole fractions and the zeolite catalyst leads to a measurable amount of N_2 formation. No ethylene was detected in the experiments with added H_2. Presumably, if it formed, it was hydrogenated to ethane.

[0131] FIG. **11** shows the yields with the different first catalysts and different conditions. The CO_2 yields are higher when heating at the first temperature and the first pressure in the reducing atmosphere than the addition of Ru/C and Ni/SiO_2—Al_2O_3 first catalysts. All other catalysts had less of an effect. The CO_2 yield for any given catalyst was generally higher in the absence rather than presence of a reducing atmosphere. The reduced CO_2 yields in the experiments with a reducing atmosphere could be due to pressure being higher in the reducing atmospheres rather than type of atmosphere. The methane yield was largely insensitive to the heating atmosphere, but was strongly dependent on catalyst type. The presence of the Ru/C and Ni/SiO_2—Al_2O_3 catalysts led to high methane yields.

[0132] The yields of N_2 were also insensitive to heating atmosphere. The yields of N_2 were low for all catalysts except the zeolite catalyst. The amount of nitrogen in FIG. **11** for the zeolite-catalyzed run corresponds to 3.3% of the nitrogen atoms originally present in the biomass solids. Thus, only a small proportion of the nitrogen atoms originally present in the biomass solids are lost to N_2. As previously discussed, the N_2 might be a product of ammonia decomposition, catalyzed by the zeolite.

[0133] The Ru/C, Pd/C, and Ni/SiO_2—Al_2O_3 catalysts led to the highest ethane yields. The ethane yields are strongly dependent on catalyst type but largely independent of the reaction atmosphere. Conversely, the ethylene yields are strongly dependent on the reaction atmosphere. Ethylene was not detected from reactions in a reducing atmosphere. The reducing atmosphere either suppressed ethylene formation or facilitated its conversion, perhaps to ethane, during the 60-minute reaction time. In inert atmospheres, all of the catalysts produced ethylene yields that were lower than that from noncatalytic reactions. The difference in ethylene yields indicates potential hydrogenation activity for the catalysts even in an inert atmosphere. Ethylene was not detected at all when the Ni/SiO_2—Al_2O_3 catalyst was present. In this regard, it may be significant that the weight percentage of active metal in the nickel catalyst is much higher (65 wt. %) than that of the other catalysts.

[0134] Tables 6 and 7 provide the ratios of carbon, hydrogen, and chemical energy (HHV) in the crude bio-oil relative to those quantities in the biomass solids heated in a reducing atmosphere or an inert atmosphere, respectively. A ratio of unity denotes recovery of the same number of atoms or amount of chemical energy in the components that were originally present in the biomass solids.

TABLE 6

Atom and Energy Balances for Heating in an Inert Atmosphere

Catalyst	C_{cb}/C_{bm}	$(C_{cb}+C_{gas})/C_{bm}$	H_{cb}/H_{bm}	$(H_{cb}+H_{gas})/H_{bm}$	E_{cb}/E_{bm}	$(E_{cb}+E_{gas})/E_{bm}$
No cat.	0.62	0.62	0.61	0.62	0.74 ± 0.01	0.75 ± 0.01
Pd/C	0.97	0.98	1.03	1.06	1.19 ± 0.04	1.21 ± 0.05
Pt/C	0.85	0.85	0.87	0.89	1.03 ± 0.09	1.04 ± 0.09
Ru/C	0.84	0.91	0.86	1.03	1.01 ± 0.05	1.14 ± 0.05
Ni/SiO_2—Al_2O_3	0.88	0.92	0.86	0.96	1.05 ± 0.06	1.12 ± 0.06
$CoMo/Al_2O_3$	0.95	0.95	0.93	0.94	1.13 ± 0.03	1.14 ± 0.03
Zeolite	0.74	0.75	0.73	0.74	0.88 ± 0.20	0.89 ± 0.20

cb—crude bio-oil

bm—wet biomass

[0135] Carbon atom recovery in the crude bio-oil increased from 62% for noncatalytic heating to greater than 90% with most of the catalysts in an inert atmosphere. Likewise, the hydrogen atom ratio for the crude bio-oil increased when catalysts were used. These higher carbon and hydrogen yields in the products led to a higher energy recovery of 90-120% for catalyzed reactions. Energy recoveries exceeding 100% are feasible when heating the wet biomass because hydrogen atoms from water can be transferred to the crude bio-oil. Table 6 also shows that the energy content of the gas above the crude bio-oil was significant only for the runs that used the Ru/C and Ni/SiO_2—Al_2O_3 catalysts. These energy recoveries, which were about 10% higher, are due to the promotion of methane formation by these catalysts.

TABLE 7

Atom and Energy Balances for Heating in a Reducing Atmosphere

Catalyst type	C_{cb}/C_{bm}	$(C_{cb} + C_{gas})/C_{bm}$	H_{cb}/H_{bm}	$(H_{cb} + H_{gas})/(_{bm} + H_{2\,feed})$	E_{cb}/E_{bm}	$(E_{cb} + E_{gas})/(E_{bm} + E_{H2\,feed})$
No cat.	0.81	0.81	0.82	1.08	0.98 ± 0.11	0.96 ± 0.08
Pd/C	0.76	0.76	0.78	1.04	0.92 ± 0.05	0.92 ± 0.04
Pt/C	0.91	0.91	0.95	1.17	1.11 ± 0.03	1.06 ± 0.02
Ru/C	0.67	0.73	0.70	1.08	0.82 ± 0.28	0.91 ± 0.21
Ni/SiO_2—Al_2O_3	0.72	0.76	0.73	1.06	0.87 ± 0.03	0.92 ± 0.02
$CoMo/Al_2O_3$	0.77	0.77	0.77	1.05	0.93 ± 0.20	0.93 ± 0.14
Zeolite	0.70	0.70	0.71	1.01	0.84 ± 0.34	0.86 ± 0.25

cb—crude bio-oil
bm—wet biomass

[0136] Heating in the reducing atmosphere (Table 7) showed little improvement in carbon, hydrogen, or energy recovery in the presence of the catalysts, although energy recovery was 98% even in the absence of catalysts. The energy recovery was generally lower for heating in the presence of the catalysts in the reducing atmosphere than in the inert atmosphere. The ratio of hydrogen in the crude bio-oil to hydrogen in the wet biomass was not increased by the presence of a reducing atmosphere, except for the case of non-catalytic heating in the presence of the Pt/C catalyst. Although, the elemental analysis showed that the crude bio-oil produced under the reducing atmosphere did have higher hydrogen content. But, since the crude bio-oil yields were generally lower in the reducing atmosphere, the total hydrogen atom recovery was also lower.

[0137] Table 7 also shows the atom and energy balances with the gas above the crude bio-oil included. The carbon and hydrogen recoveries when including the gas above the crude bio-oil are largely identical to those for the crude bio-oil alone except for the runs with the Ru/C and Ni/SiO_2—Al_2O_3 catalysts. The Ru/C and Ni/SiO_2—Al_2O_3 catalysts promoted methane formation, so the carbon and hydrogen recoveries are higher with these catalysts when including the gas above the crude bio-oil. The fourth column in Table 7 shows the ratio of hydrogen atoms in the crude bio-oil and the gas above the crude bio-oil (including those in the unreacted H_2) to hydrogen atoms in the biomass solids and H_2 initially charged to the crude reactor. This ratio exceeds unity for all runs. This result is consistent with water molecules being active participants in the chemistry, and hydrogen atoms from water being incorporated into the crude bio-oil.

[0138] The upgrading reactor, as illustrated in FIG. **2**, may be used for heating the crude bio-oil and water at the second temperature and the second pressure to form the upgraded bio-oil. In one embodiment, the upgrading reactor comprises a 400 mL autoclave and an electrical heater surrounding the autoclave. The autoclave reactor is equipped with a mixer, a mixer controller, a cooling circuit, safety relief valve, and a pressure gauge. The upgrading reactors are designed for the recovery and analysis of both crude bio-oil and the gas above the upgraded bio-oil in a single run.

[0139] In one example, 120 mL of the microalgae paste with additional water (about 80 wt. % water) is placed in the upgrading reactor to produce about 8 g of crude bio-oil. Air inside the upgrading reactor is displaced with pressurized hydrogen gas and then the upgrading reactor is charged with hydrogen at 3.4 MPa (room temperature) to provide the reducing atmosphere and to prevent water from vaporizing within the upgrading reactor. The upgrading reactor is sealed and a reaction is initiated by turning the electrical heater on and heating the wet biomass at the first temperature and the first pressure. The upgrading reactor is heated slowly for 4 hours until the upgrading reactor reaches a temperature of 320° C. (first temperature). The heating step ceased is quenched by running cool water through the internal cooling coils.

[0140] Once the upgrading reactor is cool, the pressure in the upgrading reactor is reduced to atmospheric pressure. Once cooled, the upgrading reactor has a tar-like material floating on the surface of an aqueous phase. This tar-like material is the crude bio-oil. Dichloromethane is added to the upgrading reactor to dissolve and separate any crude bio-oil from the remaining biomass solids and water. The dichloromethane extract is filtered and the dichloromethane vaporized using a rotary evaporator at 30° C. under vacuum. Any remaining material is the crude bio-oil.

[0141] The produced crude bio-oil and water may then be heated to form the upgraded bio-oil. The upgrading experiments may also carried out in the upgrading reactors shown in FIG. **2**. Typically, an amount of the crude bio-oil (0.6 g), a Pt/C catalyst (second catalyst) (0.15 g when used), and freshly deionized water (0.6 ml) are loaded into the upgrading reactor. These loadings result in a nominal water density of 0.15 g/cm^3 at super-critical conditions, a second catalyst loading of 25 wt. % relative to the crude bio-oil, and the crude bio-oil to water mass ratio of 1:1. The upgrading reactor was sealed with a cap assembly including a high pressure value. Air inside the upgrading reactor was displaced with helium (inert atmosphere) by repeatedly applying vacuum (−86 kPa gauge) and charging with helium (70 kPa gauge). Several

cycles of vacuum/charging are conducted to ensure complete removal of the air inside the upgrading reactor. The upgrading reactor is then pressurized to 70 kPa (gauge) with helium and in some instances further charged with hydrogen to 3.4 MPa (reducing atmosphere). After helium, and in some instances hydrogen, are loaded, the high-pressure valve of the upgrading reactor is securely tightened and the upgrading reactor is disconnected from any source of gas.

[0142] The upgrading reactor is placed vertically in a preheated Techne Fluidized Sand Bath (model SBL-2) with temperature control to within ±2° C. The upgrading reactor is then heated until a final temperature of 400° C. is reached. The upgrading reactor remains in the isothermal sand bath such that the upgrading reactor is maintained at the second temperature and the second pressure for 4 hours. After the step of heating the crude bio-oil and water to form the upgraded bio-oil is complete, the upgrading reactor is then removed from the sand bath, immersed in an ice-water bath for about 5 minutes to quench the reaction, further cooled in a refrigerator for 30 minutes, and kept at room temperature for at least 15 hours to allow the upgraded bio-oil to equilibrate.

[0143] After the gas above the upgraded bio-oil is analyzed using a procedure described in greater detail below, the upgrading reactor is opened. An amount of dichloromethane (3 mL) is added to the upgraded bio-oil and then the contents of the upgrading reactor are then transferred to a separatory funnel. The upgrading reactor is washed twice more with an additional 3 mL aliquot of dichloromethane to ensure that the contents of the upgrading reactor are completely collected and transferred to the separatory funnel. Once the contents of the upgrading reactor are transferred to the separatory funnel, the contents separate into an organic and an aqueous phase. The organic phase is withdrawn and the aqueous phase is extracted with sequential 5 mL and 3 mL aliquots of dichloromethane. All of the dichloromethane extracts were combined, and the second catalyst (if added) is recovered by filtering the solution. The dichloromethane is evaporated under a vacuum of 27 kPa at 30° C. for 5 minutes. Any remaining material is the upgraded bio-oil. The mass of the upgraded bio-oil is determined gravimetrically.

[0144] The gas above the upgraded bio-oil is analyzed with an Agilent Technologies model 6890N gas chromatograph (GC) equipped with a thermal conductivity detector (TCD). A 15-ft×⅛-inches i.d. stainless steel column, packed with 60×80 mesh Carboxen 1000 (Supleco) separates each component in the mixture. Argon (15 ml/min) serves as the carrier gas for the analysis. A mole fraction of each constituent in the gas above the upgraded bio-oil is determined via calibration curves generated from analysis of the analytical gas standards with known compositions. The amount of helium added to the upgrading reactor is used as an internal standard to determine the number of moles of each constituent. The yield of each constituent is calculated by dividing the number of moles of the constituent by the mass of the crude bio-oil loaded into the reactor.

[0145] An Agilent Technologies 6890N GC equipped with an auto-sampler, an auto-injector, and a mass spectrometric detector was used to analyze the upgraded bio-oil. An Agilent HP-5 capillary column (30 m length, 0.250 mm i.d., 0.10 μm film thickness) separates the constituents of the upgraded bio-oil. A sample of the upgraded bio-oil (a 2 μL volume) is dissolved in dichloromethane in a splitless mode. The capillary column is held at an initial temperature of 40° C. for 4 minutes. The initial temperature is ramped to 300° C. at 4° C./min and held at 300° C. for 10 minutes. Helium serves as a carrier gas (20 mL/min). A Wiley mass spectral library is used for constituent identification.

[0146] ^{13}C NMR spectra of the upgraded bio-oil is recorded at 100.6 MHz on a 400-MHz NMR spectrometer (Inova 400, Varian) at 25° C. A sample of the upgraded bio-oil (about 8.0 wt. % bio-oil) is dissolved in deuterochloroform ($CDCl_3$). About 10^5 scans are accumulated for the ^{13}C spectrum using a 45° pulse width together with broadband proton decoupling. Tubes of 5 mm diameter are used.

[0147] The elemental compositions (carbon, hydrogen, oxygen, nitrogen, and sulfur) of the wet biomass, the crude bio-oil, and the upgraded bio-oil are determined by Atlantic Microlab, Inc. The HHV of the upgraded bio-oil is estimated using the Dulong formula provided below:

$$\text{Heating Value (MJ/kg)} = 0.338C + 1.428(H - O/8) + 0.095S$$

wherein C, H, O, and S represent the wt % of each atom in the material.

[0148] Fourier transform infrared (FT-IR) spectroscopic analysis is performed on a Nicolet 6700 FT-IR Spectrometer (Thermo Electron Corporation) to determine functional groups of the upgraded bio-oils. FT-IR spectra (resolution: 4 cm^{-1}, scan: 254, range: 4,000-400 cm^{-1}) are taken after spreading a thin film of the upgraded bio-oil on a multi-bounce plate of ZnSe crystal at a controlled ambient temperature (25° C.). A background spectrum is also collected under identical conditions.

[0149] The TAN indicates whether a given fuel may be corrosive. The TAN value of a fuel is determined as an amount of hydroxide in milligrams that is required to neutralize acids in one gram of oil. To measure the TAN of the crude bio-oil and the upgraded bio-oil, 0.05 g of each of the crude bio-oil and the upgraded bio-oil is dissolved in acetone along with phenolphthalein, which is dissolved in methanol (0.5%, wt./v). 0.1M NaOH is added dropwise from a burette until there is a permanent color change in the sample. Volumes of NaOH added are recorded and used to calculate the TAN. The pH of the reactor contents is varied by using either HCl (0.1 M) or NaOH (0.1 M) as a reactor solution.

[0150] GC-MS analysis of the crude bio-oil reveals the presence of constituents listed in the column labeled "CBF" in Table 8. Many of the peak identities are tentatively based on the inspection of the MS fragmentation patterns and the patterns best matches with mass spectra stored in a computer library. Constituents are only listed which have at least 1.0% of the total area % of the total ion chromatogram for either the crude or one of the upgraded bio-oils (RBF). Overall, more than 100 peaks appeared in the total ion chromatogram, and the constituents identified account for more than 80% of the total peak area.

Comparison of the Composition of the Upgraded Bio-Oil and the Crude Bio-Oil

[0151] Table 8 summarizes the GS-MS identification of the major constituents in the crude and upgraded bio-oils produced under the different reaction conditions. GC-MS analysis of the crude bio-oil reveals the presence of constituents listed in the column labeled "CBF" in Table 8. Many of the peak identities are tentatively based on the inspection of the MS fragmentation patterns and the patterns best matches with mass spectra stored in a computer library. Constituents are only listed which have at least 1.0% of the total area % of the total ion chromatogram for either the crude or one of the upgraded bio-oils (RBF). Overall, more than 100 peaks appeared in the total ion chromatogram, and the constituents identified account for more than 80% of the total peak area.

TABLE 8

Tentative Identities and Area % of Major Peaks in Total Ion Chromatograms for Crude and Upgraded Bio-oils

Retention Time in GC (min)	Compound	CBF Area %	RBF Pt/C H_2 Area %	RBF No Cat H_2 Area %	RBF Pt/C No H_2 Area %	RBF Pt/C H_2, HCl Area %	RBF Pt/C H_2, NaOH Area %
5.73	Toluene	0.48	3.32	2.93	3.48	2.80	2.80
9.07	Ethyl-benzene	0.20	2.40	2.56	1.83	1.81	2.22
19.22	Undecane	—	1.09	0.56	1.24	0.99	1.09
23.18	Dodecane	—	1.31	0.72	1.50	1.22	1.30
26.93	Tridecane	0.19	2.39	1.25	2.19	2.66	2.37
28.57	1-Methyl piperidine	1.10	—	0.29	—	0.15	—
30.43	Tetradecane	—	2.12	1.66	2.64	1.96	2.93
33.31	2,7-Dimethyl-indolizine	—	0.14	1.23	0.87	0.67	0.73
33.80	Pentadecane	0.15	12.36	7.93	11.10	9.71	13.39
36.93	Hexadecane	—	4.24	1.87	2.19	4.55	5.28
38.35	2,6,10-Trimethyl-pentadecane	—	0.72	0.94	0.87	0.94	1.03
39.88	Heptadecane	0.55	5.01	2.41	3.50	3.90	4.26
40.03	2,6,10,14-Tetramethyl-pentadecane	0.17	1.01	0.82	0.91	0.86	1.01
41.78	Myristic acid	1.69	0.40	1.56	1.16	0.75	0.35
42.67	Octadecane	0.21	2.14	0.87	0.87	2.10	1.94
42.98	2,6,10,14-Tetramethyl-hexadecane	0.99	5.30	4.88	2.91	4.21	4.49
43.55	2-Phytene isomer	1.11	—	—	—	—	—
43.93	2-Phytene isomer	3.64	—	—	—	—	—
45.36	Nonadecane	0.22	3.23	4.05	3.35	2.98	3.01
46.85	Palmitoleic Acid	6.82	—	—	—	—	—
47.35	Palmitic acid	14.23	3.84	12.02	6.30	4.18	2.86
47.47	Myristamide	—	0.75	1.68	0.79	0.79	0.57
47.75	1-Methyl-9H-pyrido[3,4-b] indole	1.27	—	—	—	—	—
47.90	Eicosane	—	0.67	0.99	0.64	0.60	0.47
52.36	Palmitamide	3.52	4.09	5.59	7.05	4.15	4.89
53.14	Decanal, O-methyloxime	2.46	—	—	—	—	—
54.07	N,N-Dimethylpalmitamide	2.02	—	—	—	—	—
56.86	Stearamide	0.31	0.53	0.84	1.27	0.86	0.80
57.07	Tetracosane	3.13	0.82	0.58	0.81	0.65	0.64
58.90	1-Methyl-naphthalene	1.28	—	—	—	—	—
59.01	1-[(2-propenyloxy)methyl] Naphthalene	1.10	—	—	—	—	—
59.14	Pentacosane	0.66	0.98	0.98	1.10	1.03	0.96
59.40	Cholesterol	8.72	—				
60.87	1-(1-pyrrolidinyl)-1-Octadecanone	2.78	—	—	—	—	—
61.14	Hexacosane	0.63	0.86	0.89	1.10	0.98	0.90
63.07	Heptacosane	1.27	2.26	2.15	2.17	1.90	2.03
63.31	1-Methyl-2-Piperidinethione,	1.14	—	—	—	—	—
64.93	Octacosane	0.61	1.68	1.52	1.53	1.49	1.40
66.02	Cholest-4-ene	2.39	—	—	—	—	—
66.23	Cholestane	2.80	0.62	0.68	0.49	0.36	0.49
66.73	Nonacosane	0.96	2.56	2.13	2.28	1.81	2.23
68.46	Triacontane	0.31	2.25	1.69	1.85	2.02	1.89
70.17	Hentriacontane	0.27	2.13	1.30	1.49	1.96	1.70
72.22	Dotriacontane	0.25	5.35	3.61	3.72	4.55	4.03
	Total	69.6	75.9	72.2	72.6	69.0	73.6

[0152] The relative content of alkane products in the upgraded bio-oils was much higher than in the crude bio-oil. Pentadecane was the most abundant alkane in the upgraded bio-oils, but other alkanes ranging from C_9 to C_{33} are also present. Alkanes smaller than C_{15} were not detected in appreciable amounts, so lighter molecules in the upgraded bio-oil may have formed from cracking of longer chain alkanes during the step of heating the crude bio-oil and water at the second temperature and the second pressure. As an example, in the upgraded bio-oil formed in the presence of the Pt/C catalyst and a reducing atmosphere, alkane peaks in the total ion chromatogram account for fully 68% of the total area. Alkenes were present in the crude bio-oil which the upgraded bio-oil is derived from, but no alkenes were detected in the upgraded bio-oil. The alkenes may have been hydrogenated. In addition to the formation of alkanes, perhaps through cracking and/or hydrogenation, significant amounts of alkyl substituted benzenes are formed as well. Derivatives of piperidine, indole and O-methyloxime, which were present in the crude bio-oil, are not detected in the upgraded bio-oil. This suggests that the catalyst and conditions used resulted in denitrogenation. The overall content of cholesterol, cholestane, and cholestene decreased in the upgraded bio-oil as compared to crude bio-oil. Furthermore, the only sulfur-containing compound, 1-methyl-2-piperidinethione, detected in the crude bio-oil is not present in the upgraded bio-oil.

[0153] The difference in the molecular compositions of the crude bio-oil and upgraded bio-oil is evident in FIG. **12**, which compares the total ion chromatograms for each. The chromatogram for the crude bio-oil shows little material eluting prior to 40 min, and it shows a large, broad signal around 48 min that corresponds to fatty acids. In contrast, the

upgraded bio-oil shows large peaks at retention times shorter than 12 min and many regularly spaced peaks, which correspond to a series of n-alkanes starting at about C_9.

[0154] Table 9 compares the elemental compositions and TAN of the crude bio-oil and upgraded bio-oils produced under different reaction conditions. The crude bio-oil has an elemental composition of 77.3 wt. % C; 10.5% H; 6.52% O; 4.89% N; and 0.68% S. Chlorine was present in trace amounts (less than 0.25%), as is reasonable for a wet biomass comprising a marine algae. The heating value of the crude bio-oil is about 40.1 MJ/kg, which is higher than that of bio-oils obtained from pyrolysis of microalgae, and is close to that of petroleum-derived crudes (about 42 MJ /kg). The crude bio-oil has a high total acid number of 256, indicating the potential for corrosion problems and confirming the necessity of upgrading the crude bio-oil.

TABLE 9

Elemental Composition (wt. %) and TAN of Crude and Upgraded Bio-oils

	CBF	RBF (Pt/C, H_2)	RBF (no cat, H_2)	RBF (Pt/C, no H_2)	RBF (Pt/C, H_2, HCl)	RBF (Pt/C, H_2, NaOH)
TAN	256.5	25.3	49.6	71.2	41.1	25.6
C	77.32	82.09	82.75	80.26	81.96	81.56
H	10.52	11.21	10.76	10.91	11.05	11.34
O	6.52	4.46	4.31	4.68	4.32	4.71
N	4.89	2.24	2.17	2.79	2.76	2.38
S	0.68	n.d.	n.d	n.d	n.d	n.d
Cl	<0.25	n.d	n.d	n.d	n.d	n.d
H/C	1.63	1.64	1.56	1.63	1.62	1.67
O/C	0.063	0.041	0.039	0.044	0.040	0.043
N/C	0.054	0.023	0.022	0.030	0.029	0.025
Heating value (MJ/kg)	40.1	43.0	42.6	41.9	42.7	42.9

n.d. = none detected

FT-IR Analysis of Crude and Upgraded Bio-Oil

[0155] FIG. **13** shows the FT-IR spectra for the crude and one of the upgraded bio-oil produced. All of the upgraded bio-oil have similar FT-IR spectra, which suggest that the same types of functional groups exist in each. Both the crude and upgraded bio-oil display strong absorbance between 2850 and 3000 cm^{-1}, indicating a high content of ethylene groups. This result is consistent with the presence of a collection of fatty acids and alkanes in the total ion chromatogram. Absorbance peaks between 1700 and 1760 cm^{-1} represent the C═O group stretching vibration in carboxylic acids. The upgraded bio-oils show a proportionately lower absorbance in this wave number range, which is consistent with reduced fatty acid content.

[0156] The appearance of distinct bands between 710 and 860 cm^{-1} in the upgraded bio-oils is likely due to the presence of substituted benzenes in the upgraded bio-oil.

[0157] FIG. **14** displays the ^{13}C NMR spectrum of the upgraded oil formed in the presence of the Pt/C catalyst a reducing atmosphere (3.4 MPa H_2). A strong peak at 54 ppm corresponds to residual dichloromethane solvent. This residual solvent is likely present because of mild conditions used when removing the solvent in the rotary evaporator. The mild conditions were used in an effort to retain some of the lighter ends of the upgraded bio-oil in the sample that gets analyzed. There are numerous peaks in the 10-50 ppm region, where aliphatic methyl and methylene carbon (such as alkane carbons) atoms appear. FIG. **14** shows peaks in the 110-160 ppm range, which are indicative of alkenyl and aromatic carbon atoms. These peaks were present, but less pronounced in the NMR spectrum of the crude feed. The upgraded bio-oil shows a resonance at about 205 ppm, which is in the region where the carbonyl carbon in ketones and aldehydes appear. This peak was absent in the crude bio-oil, as was evidence for ketones or aldehydes in the total ion chromatogram. The upgraded bio-oil did not have a peak until about 180 ppm, which is where carbon atoms in carboxylic acids appear. The absence of this peak is consistent with the greatly reduced level of fatty acids in the upgraded bio-oil.

Distribution of Gas Above the Upgraded Bio-Oil

[0158] Constituents of the gas above the upgraded bio-oil were formed in low yields. The total amount of gas above the upgraded bio-oil ranged from a low of 1.5 wt % (no catalyst, reducing atmosphere) to a high of 2.7 wt. % (Pt/C, inert atmosphere). When a reducing atmosphere is present during the step of heating the crude bio-oil and water at the second temperature and the second pressure, CH_4, C_2H_6, and CO_2 were, by far, the most abundant gases formed. FIG. **15** shows the yield of each of these gases for the different conditions, including the case of an inert atmosphere. The step of heating the crude bio-oil and water at the second temperature and the second pressure with the water being acidic and a reducing atmosphere provided the highest yields of each of the three gases. The yields of gas above the upgraded bio-oil are always lowest in uncatalyzed reactions. This result is reasonable, especially for the CO_2 yield, because the Pt/C catalyst is a good catalyst for hydrothermal decarboxylation of fatty acids. Interestingly, the yields of the gas above the upgraded bio-oil formed in the presence of the Pt/C catalyst in an inert atmosphere exceeded yields obtained when a reducing atmosphere is present. It is possible that the higher reaction pressure in the reducing atmosphere inhibited gas formation during the step of heating the crude bio-oil and water at the second temperature and the second pressure. In addition to the three main gaseous products in FIG. **15**, CO and ethylene formed in low yields in some experiments. The upgraded bio-oils formed in the absence of either the Pt/C catalyst or a reducing atmosphere led to modest CO production (~0.01 mmol/g). The upgraded bio-oil formed in the absence of the Pt/C catalyst is the only one to produce a measurable yield of

ethylene (~0.005 mmol/g). Presumably, any ethylene formed would be hydrogenated to ethane when the Pt/C catalyst is present.

[0159] FIG. **16** displays data for H_2 consumption from the step of heating the crude bio-oil and water at the second temperature and the second pressure. Hydrogen consumption under these conditions is modestly higher when the Pt/C catalyst is present. A possible reason for this outcome is the presence of the second catalyst facilitating hydrogenation and hydrodeoxygenation paths.

[0160] No nitrogen (N_2) was detected the upgraded bio-oil, which is consistent with the theory that nitrogen atoms released from the crude bio-oil during the step of heating at the second temperature and the second pressure in the form of ammonia. Testing the pH of the aqueous phase after the step of heating the crude bio-oil and water at the second temperature and the second pressure revealed that the aqueous phase was basic, and ammonia was detected via GC-MS analysis of the aqueous phase.

Mass and Energy Balance

[0161] Overall recoveries of carbon and hydrogen atoms and heating value from the step of heating at the second temperature and the second pressure is calculated from the elemental compositions of the crude and upgraded bio-oil and the yields of the gas above the crude bio-oil and upgraded bio-oil formed. The results show that about 82 wt. % of the carbon and 74 wt. % of the hydrogen atoms in the crude bio-oil and H_2 initially loaded into the reactor were recovered in the upgraded bio-oil. Moreover, about 3.0 wt. % of the carbon and 5.8 wt. % of the hydrogen appeared in the gas above the upgraded bio-oil. Thus, the upgraded bio-oil and the gas above the upgraded bio-oil include about 85% and 80% of the carbon and hydrogen atoms, respectively, originally loaded to the reactor in the form of the biomass solids. The "missing" carbon and hydrogen could be in compounds that selectively partitioned into the aqueous phase or, more likely, in light organic compounds that evaporated from the upgraded bio-oil during the rotary-vaporization process used to recover the upgraded bio-oil.

[0162] The upgraded bio-oil and the gas above the upgraded bio-oil recover 83% of the energy present in the crude bio-oil and H_2 originally loaded into the upgrading reactor. Chemical energy was probably lost along with the residual organics in the aqueous phase and light products lost during the step of heating the crude bio-oil and water at the second temperature and the second pressure.

[0163] It is to be understood that the appended claims are not limited to express particular compounds, compositions, or methods described in the detailed description, which may vary between particular embodiments that fall within the scope of the appended claims. With respect to any Markush groups relied upon herein for describing particular features or aspects of various embodiments, it is to be appreciated that different, special, and/or unexpected results may be obtained from each member of the respective Markush group independent from all other Markush members. Each member of a Markush group may be relied upon individually and/or in combination and provides adequate support for specific embodiments within the scope of the appended claims.

[0164] It is also to be understood that any ranges and subranges relied upon in describing various embodiments of the present invention independently and collectively fall within the scope of the appended claims and are understood to describe and contemplate all ranges, including whole and/or fractional values therein, even if such values are not expressly written herein. One of skill in the art readily recognizes that the enumerated ranges and subranges sufficiently describe and enable various embodiments of the present invention and such ranges and subranges may be further delineated into relevant halves, thirds, quarters, fifths, and so on. As just one example, a range "ranging from 0.1 to 0.9" may be further delineated into a lower third, i.e., from 0.1 to 0.3, a middle third, i.e., from 0.4 to 0.6, and an upper third, i.e., from 0.7 to 0.9, which individually and collectively are within the scope of the appended claims and may be relied upon individually and/or collectively and provide adequate support for specific embodiments within the scope of the appended claims.

[0165] In addition, with respect to the language which defines or modifies a range, such as "at least," "greater than," "less than," "no more than," and the like, it is to be understood that such language includes subranges and/or an upper or lower limit. As another example, a range of "at least 10" inherently includes a subrange ranging from at least 10 to 35, a subrange ranging from at least 10 to 25, a subrange ranging from 25 to 35, and so on, and each subrange may be relied upon individually and/or collectively and provides adequate support for specific embodiments within the scope of the appended claims. Finally, an individual number within a disclosed range may be relied upon and provides adequate support for specific embodiments within the scope of the appended claims. For example, a range "ranging from 1 to 9" includes various individual integers, such as 3, as well as individual numbers including a decimal point (or fraction), such as 4.1, which may be relied upon and provide adequate support for specific embodiments within the scope of the appended claims.

[0166] The invention has been described in an illustrative manner and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation. Many modifications and variations of the present invention are possible in light of the above teachings and the invention may be practiced otherwise than as specifically described.

What is claimed is:

1. A method of producing an upgraded bio-oil from a wet biomass, the method comprising:

providing the wet biomass comprising water and biomass solids;

heating the wet biomass at a first temperature and a first pressure for a time period ranging from 10 to 200 minutes to form a crude bio-oil, with the first temperature ranging from 200 to 400° C. and the first pressure ranging from 0.1 to 25 MPa, with the proviso that at least a portion of the water present in the wet biomass remains in a liquid phase throughout the step of heating to form the crude bio-oil, and the first temperature and first pressure are below super-critical conditions of water; and

heating the crude bio-oil and water at a second temperature and a second pressure for a time period of at least 30 minutes to form the upgraded bio-oil, with the proviso that at least a portion of the water remains in the liquid phase or in a super-critical fluid phase throughout the step of heating to form the upgraded bio-oil, and

wherein the second temperature is greater than the first temperature and is at least 300° C.

2. The method of claim **1**, wherein the wet biomass comprises at least 70 wt. % water based on the total weight of the wet biomass.

3. The method of claim **2**, wherein the wet biomass comprises algae having a lipid content ranging from 20 to 80 wt. % based on the total weight of the biomass solids.

4. The method of claim **1**, wherein the second temperature and the second pressure are within super-critical conditions.

5. The method of claim **1**, wherein the step of heating the wet biomass is conducted in the presence of a first catalyst.

6. The method of claim **5**, wherein the first catalyst comprises a heterogeneous catalyst selected from the group consisting of Pd/C, Pt/C, Ru/C, Ni/SiO_2—Al_2O_3, sulfided $CoMo/\gamma$-Al_2O_3, zeolite, activated carbon, and combinations thereof.

7. The method of claim **5**, wherein the step of heating the crude bio-oil and water is conducted in the presence of the first catalyst.

8. The method of claim **5**, wherein the step of heating the crude bio-oil and water is conducted in the presence of a second catalyst selected from the group consisting of Pd/C, Pt/C, Ru/C, Ni/SiO_2—Al_2O_3, sulfided $CoMo/\gamma$-Al_2O_3, zeolite, activated carbon, and combinations thereof, wherein the first catalyst is different from the second catalyst.

9. The method of claim **1**, wherein the step of heating the wet biomass is conducted in a reducing atmosphere.

10. The method of claim **1**, wherein the step of heating the wet biomass is conducted in an inert atmosphere.

11. The method of claim **1**, wherein the step of heating the crude bio-oil and water is conducted in an inert atmosphere.

12. The method of claim **1**, wherein the step of heating the crude bio-oil and water is conducted in a reducing atmosphere.

13. The method of claim **1**, wherein the crude bio-oil comprises an amount of carbon ranging from 50 to 80 wt. % of the crude bio-oil, an amount of hydrogen ranging from 1 to 20 wt. % of the crude bio-oil, an amount of oxygen ranging from 5 to 20 wt. % of the crude bio-oil, an amount of nitrogen ranging from 1 to 15 wt. % of the crude bio-oil, and an amount of sulfur ranging from 0 to 5 wt. % of the crude bio-oil.

14. The method of claim **1**, wherein the upgraded bio-oil comprises an amount of carbon ranging from 70 to 90 wt. % of the crude bio-oil, an amount of hydrogen ranging from 1 to 20 wt. % of the crude bio-oil, an amount of oxygen ranging from 1 to 15 wt. % of the crude bio-oil, an amount of nitrogen ranging from 1 to 15 wt. % of the crude bio-oil, and an amount of sulfur less than 1 wt. % of the crude bio-oil.

15. The method of claim **1**, wherein the step of heating the wet biomass is conducted for a duration ranging from 55 to 100 minutes and wherein the step of heating the crude bio-oil and water is conducted for a duration ranging from 60 to 300 minutes.

16. An upgraded bio-oil obtained from the refinement of crude bio-oil produced by the hydrothermal liquefaction of wet biomass, the upgraded bio-oil comprising:

carbon in the amount ranging from 70 to 90 wt. %;

hydrogen in an amount ranging from 1 to 20 wt. %;

oxygen in an amount ranging from 1 to 15 wt. %;

nitrogen in an amount ranging from 1 to 15 wt. %;

sulfur in an amount of less than 1 wt. %, all based on the total weight of the upgraded bio-oil, with the proviso that the heating value of the upgraded bio-oil ranges from 35 to 55 MJ/kg.

17. A method of producing an upgraded bio-oil from a wet biomass using hydrothermal liquefaction, the method comprising:

providing the wet biomass comprising at least 70 wt. % water and less than 30 wt. % biomass solids based on the total weight of the wet biomass;

heating the wet biomass at a first temperature and a first pressure for a time period ranging from 10 to 200 minutes to form a crude bio-oil, with the first temperature ranging from 200 to 400° C. and the first pressure ranging from 0.1 to 25 MPa, with the proviso that at least a portion of the water present in the wet biomass remains in a liquid phase throughout the step of heating to form the crude bio-oil, and the first temperature and pressure are below super-critical conditions for the water; and

heating the crude bio-oil and the water at a second temperature and a second pressure for a time period of at least 30 minutes to form the upgraded bio-oil, with the proviso that at least a portion of the water remains in a liquid phase or in a super-critical fluid phase throughout the step of heating to form the upgraded bio-oil, and

wherein the second temperature is greater than the first temperature and at least 300° C.

18. The method of claim **17**, wherein the upgraded bio-oil comprises an amount of carbon ranging from 70 to 90 wt. % of the crude bio-oil, an amount of hydrogen ranging from 1 to 20 wt. % of the crude bio-oil, an amount of oxygen ranging from 1 to 15 wt. % of the crude bio-oil, an amount of nitrogen ranging from 1 to 15 wt. % of the crude bio-oil, and an amount of sulfur less than 1 wt. % of the crude bio-oil.

19. The method of claim **17**, wherein the step of heating the wet biomass is conducted in the presence of a first catalyst comprising a heterogeneous catalyst selected from the group consisting of Pd/C, Pt/C, Ru/C, Ni/SiO_2—Al_2O_3, sulfided $CoMo/y$-Al_2O_3, zeolite, activated carbon, and combinations thereof.

20. The method of claim **17**, wherein the step of heating the wet biomass is conducted in a reducing atmosphere.

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