

**VALORIZATION OF INVASIVE PLANT SPECIES TO
PRODUCE BIO-CHEMICALS AND BIO-PRODUCTS
THROUGH GREENER APPROACHES**

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**CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
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by

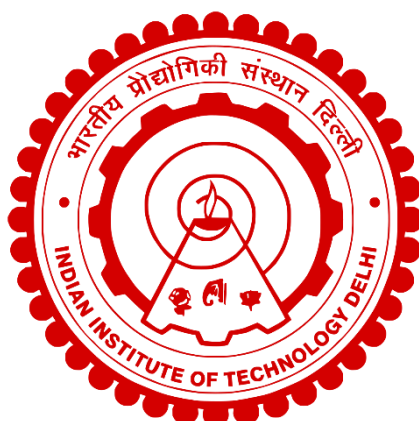
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Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the



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“Dedicated to My Apā”

CERTIFICATE

This is to certify that the thesis entitled “**Valorization of Invasive Plant Species to Produce Bio-chemicals and Bio-products Through Greener Approaches**”, being submitted by Mr. Falguni Pattnaik to the Indian Institute of Technology Delhi for the award of “Doctor of Philosophy” is a record of bonafide research work carried out by her. He has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of our knowledge, the results contained in this thesis have not been submitted in part or full to any other university or institute for the award of any degree or diploma.

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ABSTRACT

This Ph.D. work is comprised of the valorization of two highly invasive under-utilized lignocellulosic biomasses such as *Phragmites karka* and *Cannabis indica* for different biorefinery applications like extraction of medicinal molecules, conversion of polysaccharides into platform chemicals and isolation of cellulose fibre using various greener techniques like supercritical CO₂ (SCCO₂) extraction, subcritical water (SbCW) assisted catalytic and pulping-bleaching processes, respectively. Prior to conducting various applications, the biomasses were physicochemically characterized to assess the potentiality of the biomasses towards various applications. By evaluating the physicochemical properties, it was determined that both the biomasses consisted of higher cellulosic contents with a very low lignin contents (For *Phragmites karka* lignin content: 14.6 wt.% and for *Cannabis indica*: 14.3 wt.%), which decreases the recalcitrance of both the biomasses establishing an application of converting the hemicelluloses into platform chemicals and isolating cellulose fibres. Besides these applications, the leaves of *Cannabis indica* constitute various cannabinoids, which was conventionally used for various medicinal purposes.

The leafy parts of *Cannabis indica*, an under-utilized perennial weed was taken as the feedstock to extract cannabinoids using supercritical CO₂ (SCCO₂) extraction through central composite design (CCD) by taking CO₂ pressure, temperature and extraction time as the experimental parameters. Among these three parameters, CO₂ pressure had a greater impact on the extraction process than the other two factors out of all the variables. With a CO₂ pressure of 250 bar, a vessel temperature of 40 °C, and a 1.5 h extraction period, the largest and most acceptable yield of cannabis oil of 4.82 wt.% was obtained. The contents included in the cannabis oil and their structural properties were determined using FTIR (Fourier-Transform Infrared Spectroscopy), ¹H NMR (Proton Nuclear Magnetic Resonance) spectroscopy, and GC-MS (Gas chromatography-Mass spectrophotometry). The extract contains four primary cannabinoids, including CBD (Cannabidiol), THCV (Tetrahydrocannabivarin), Δ⁹-THC (Δ⁹-Tetrahydrocannabinol), and Δ⁸-THC (Δ⁸-Tetrahydrocannabinol), as well as two distinctive terpenoids: cis-caryophyllene and α-humulene. The residue biomass generated from the SCCO₂ extraction was employed as the feedstock for polyphenol extraction utilizing water as the solvent. Apart from accessing the structural features of the various cannabis extractives, the DPPH (2,2-Diphenyl-1-picrylhydrazyl)

assay or anti-oxidant activity of the cannabis oil and water extractive was evaluated, with IC₅₀ (Half-maximal inhibitory concentration) values of 1.3 and 0.6 mg/mL, respectively, which can be compared to the commercially available anti-oxidant BHT (Butylated hydroxy toluene), which has an IC₅₀ value of 0.5 mg/mL.

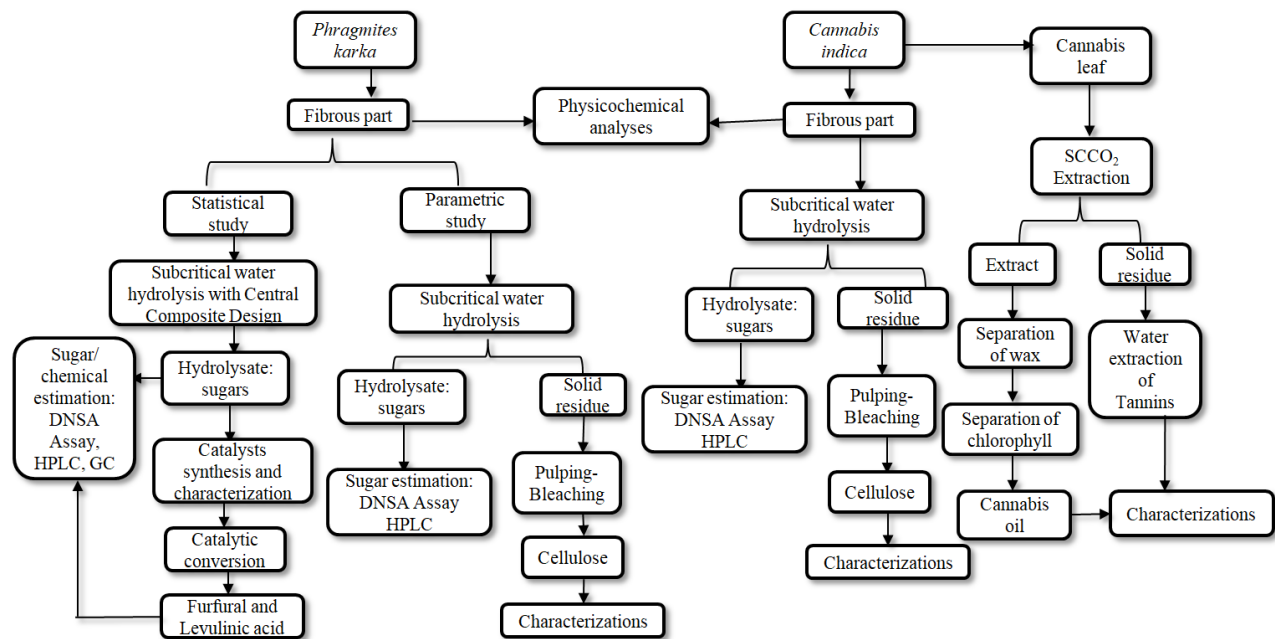
Besides the leafy part of the biomass, fibrous stem part was used to extract sugars and cellulose fibre through the integrated subcritical water hydrolysis assisted pulping-bleaching process. Preliminarily, the feedstock was treated with subcritical water through the statistical designing of the experiments using central composite design (CCD) by taking temperature, reaction time and feed concentration as the experimental variables in the range of 150-230 °C, 15-60 min and 2-5 wt.%. The hydrolysate obtained from the subcritical water hydrolysis was analyzed for the total reducing sugars (TRS) and individual sugars yields. The highest TRS yield was found to be 16.4 wt.% at the temperature, reaction time and feed concentration of 190 °C, 37.5 min and 3.5 wt.%, respectively. The optimized conditions were deduced from the statistical model and the solid residue obtained from the optimized conditions was treated with 0.5 M NaOH and 0.5-3% hydrogen peroxide to isolate cellulose fibre, whose yield was found to be 34.8 wt.% with the lignin content of 0.5 wt.%.

Phragmites karka, also known as common reed, is a perennial grass and a highly invasive crop species, which creates ecological problems by competing with native biodiversity and vegetation. This study involves subcritical water hydrolysis of *Phragmites* to produce monomeric sugars followed by the catalytic conversion of the sugar-rich hydrolysate to furfural and levulinic acid. Subcritical water hydrolysis was performed by the Central Composite Design method at variable temperatures (150–230 °C), reaction time (15–60 min) and feed concentration (2–5 wt.%). The temperature was found to be the most prominent factor affecting biomass hydrolysis. The yield of total reducing sugars from biomass hydrolysis was in the range of 2.1–18.1 % where the highest yield was obtained at the optimal temperature (190 °C), reaction time (37.5 min) and feed concentration (2 wt.%). During subcritical water hydrolysis of *Phragmites*, two main degradation products obtained at a higher temperature (230 °C) and reaction time (37.5 min) were furfural (8.2 wt.%) and 5-hydroxymethylfurfural (11.7 wt.%). However, at 230 °C and a longer reaction time of 60 min, 5-hydroxymethylfurfural yield reduced to 5.1 % owing to its conversion to humin while furfural yield elevated to 9.9 wt.%. Catalysts such as ZrO₂, TiO₂, Zr_{0.5}Ti_{0.5}O₂, WO₃-ZrO₂, WO₃-

TiO₂ and WO₃-Zr_{0.5}Ti_{0.5}O₂ were involved in the conversion of the sugar-rich hydrolysate obtained from subcritical water hydrolysis of *Phragmites*. The highest sugar conversion was found to be 92% with WO₃-ZrO₂ resulting in the yields of furfural (51%) and levulinic acid (34%). The activity of particular catalysts (e.g., WO₃-ZrO₂, WO₃-TiO₂ and WO₃-Zr_{0.5}Ti_{0.5}O₂) relied on the synergistic effects of Lewis and Brønsted acid sites. Furthermore, this feedstock was also used to isolate cellulose fibre using a greener and integrated technique. Cellulose fibers were isolated from wetland reed grass (*Phragmites karka*) by consolidated subcritical water (SbCW) hydrolysis, pulping and bleaching process. The SbCW treated biomass was carried out through pulping and bleaching process to isolate cellulose fibers. The highest cellulose yield was found to be 35.1 wt.% with a residual lignin content of 0.4 wt.% at 0.5 M NaOH.

With the inclusion of subcritical water hydrolysis, a higher yield of cellulose was obtained from *Phragmites karka* and *Cannabis indica* with a crystallinity index of 65.3% and 65%, respectively. Due to the effective hydrolysis process, subsequent processes such as pulping and bleaching required less severe conditions. Furthermore, in a comparative study, the untreated biomass, subcritical water hydrolyzed biomass and cellulose fibres were characterized by several physicochemical characterization tools such as proximate, ultimate and compositional analysis, thermogravimetric analysis, Fourier-transform infrared, Fourier-transform near-infrared and Raman spectroscopy, X-ray diffraction, ¹³C solid-state nuclear magnetic resonance spectroscopy, scanning electron microscopy, transmission electron microscopy and atomic force microscopy techniques were used to estimate the crystallinity index, carbon content, delignification, cellulose recovery as well as thermal stability and morphology of cellulose fibers. By these processes, both the invasive biomasses were utilized and opened a new research platform for these feedstocks.

Graphical abstract



सारांश

यह पीएच.डी. काम में दो अत्यधिक आक्रामक कम-उपयोग किए गए लिग्नोसेल्यूलोसिक (lignocellulosic) बायोमास जैसे कि *Phragmites karka* और *Cannabis indica* के विभिन्न हरित तकनीकों (अतिक्रांतिक कार्बन डाईऑक्साइड और उप-क्रांतिक जल विश्लेषण) का उपयोग करके विभिन्न बायोरिफाइनरी अनुप्रयोगों जैसे औषधीय अणुओं के निष्कर्षण, बहुशर्करा को प्लेटफॉर्म रसायनों (Platform chemical) में रूपांतरण और सेलूलोज़ फाइबर के अलगाव शामिल हैं। विभिन्न अनुप्रयोगों के संचालन से पहले, बायोमास को विभिन्न अनुप्रयोगों के लिए बायोमास की क्षमता का आकलन करने के लिए भौतिक-रासायनिक रूप से चित्रित किया गया था। भौतिक-रासायनिक गुणों का मूल्यांकन करके, यह निर्धारित किया गया था कि दोनों बायोमास में बहुत कम लिग्निन के साथ उच्च सेल्यूलोसिक भाग शामिल थी (*Phragmites karka* का लिग्निन भाग: १४. ६ wt.% और *Cannabis indica* का लिग्निन भाग: १४. ३ wt.%), जो जटिलता को कम करता है और दोनों बायोमास की hemicellulose को प्लेटफॉर्म रसायनों में परिवर्तित करने और सेल्यूलोज़ फाइबर को अलग करने के एक एक प्रयोग की स्थापना करते हैं। इन अनुप्रयोगों के अलावा, *Cannabis indica* की पत्तियां से विभिन्न कैनबिनोइड्स (cannabinoids) का निष्कर्षण किया हुआ है, जिनका पारंपरिक रूप से विभिन्न औषधीय प्रयोजनों के लिए उपयोग किया जाता है।

Cannabis indica के पत्तेदार हिस्सों, एक कम उपयोग किए जाने वाले बारहमासी खरपतवार को प्रायोगिक मापदंडों के रूप में CO₂ दबाव, तापमान और निष्कर्षण समय लेकर केंद्रीय समग्र डिजाइन (CCD) के माध्यम से अतिक्रांतिक कार्बन डाईऑक्साइड (SCCO₂) निष्कर्षण का उपयोग करके कैनबिनोइड्स निकालने के लिए फीडस्टॉक के रूप में लिया गया था। इन तीन मापदंडों में, सभी चरों में से अन्य दो कारकों की तुलना में CO₂ दबाव का निष्कर्षण प्रक्रिया पर अधिक प्रभाव पड़ा। २५० बार (bar) के CO₂ दबाव, ४० डिग्री सेल्सियस के एक बर्तन के तापमान और १.५ घंटे की निकासी अवधि के साथ, ४.८२ wt.% की भांग के तेल की सबसे बड़ी और सबसे स्वीकार्य उपज प्राप्त की गई थी। भांग के तेल में शामिल सामग्री और उनके संरचनात्मक गुणों को FTIR (फूरियर-ट्रान्सफॉर्म इन्फ्रारेड स्पेक्ट्रोस्कोपी), ¹H NMR (प्रोटॉन न्यूक्लियर मैग्नेटिक रेजोनेंस) स्पेक्ट्रोस्कोपी, और GC-MS (गैस क्रोमैटोग्राफी- मास स्पेक्ट्रोफोटोमेट्री) का उपयोग करके निर्धारित किया गया था। अर्क में चार प्राथमिक कैनबिनोइड्स होते हैं, जिनमें CBD (कैनाबीडियोल), THCV (टेट्राहाइड्रोकैनाबीवरिन), Δ⁹-THC (Δ⁹-टेट्राहाइड्रोकैनाबिनोल), और Δ⁸-THC (Δ⁸-टेट्राहाइड्रोकैनाबिनोल), साथ ही दो विशिष्ट टरपेनोइड्स (Terpenoids) शामिल हैं: cis- कैरियोफिलीन और α-humulene।

अतिक्रांतिक कार्बन डाईऑक्साइड (SCCO₂) निष्कर्षण से उत्पन्न अवशेष बायोमास को विलायक के रूप में पानी का उपयोग करके पॉलीफेनोल निष्कर्षण के लिए फीडस्टॉक के रूप में नियोजित किया गया था। विभिन्न भांग के अर्क की संरचनात्मक विशेषताओं तक पहुँचने के अलावा, भांग के तेल और पानी के अर्क (water extractives) के DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay से एंटी-ऑक्सीडेंट गतिविधि का मूल्यांकन IC₅₀ (आधा-अधिकतम अवरोधक एकाग्रता) के साथ किया गया था। क्रमशः १.३ और ०. ६ मिलीग्राम/ माइक्रोलीटर के मान, जिसकी तुलना व्यावसायिक रूप से उपलब्ध एंटी-ऑक्सीडेंट बीएचटी (ब्यूटाइलेटेड हाइड्रॉक्सी टोल्यूनि) से की जा सकती है, जिसका IC₅₀ मान ०. ५ मिलीग्राम/ माइक्रोलीटर है।

बायोमास के पत्तेदार हिस्से के अलावा, रेशेदार स्टेम भाग का उपयोग एकीकृत उप-क्रांतिक वाटर (जल) विश्लेषण असिस्टेड पल्पिंग-ब्लीचिंग प्रक्रिया के माध्यम से शर्करा और सेल्यूलोज फाइबर निकालने के लिए किया गया था। प्रारंभिक रूप से, फीडस्टॉक को १५० - २३० डिग्री सेल्सियस, १५-१५ मिनट की सीमा में प्रयोगात्मक चर के रूप में तापमान, प्रतिक्रिया समय और फ्रीड सांद्रता का उपयोग करके केंद्रीय समग्र डिजाइन (CCD) का उपयोग करके प्रयोगों के सांख्यिकीय डिजाइन के माध्यम से उप-क्रांतिक पानी के साथ इलाज किया गया था। और २-५ wt.%। सबक्रिटिकल वाटर विश्लेषण से प्राप्त हाइड्रोलाइज़ेट का कुल अपचायक शर्करा (TRS) और व्यक्तिगत शर्करा (sugars) की पैदावार के लिए किया गया था। तापमान, प्रतिक्रिया समय और फ्रीड सांद्रता में क्रमशः १९० डिग्री सेल्सियस, ३७.५ मिनट और ३.५ wt.% पर उच्चतम टीआरएस उपज १६.४ wt.% पाई गई। अनुकूलित स्थितियों को सांख्यिकीय मॉडल से घटाया गया था और अनुकूलित स्थितियों से प्राप्त ठोस अवशेषों को सेल्यूलोज फाइबर को अलग करने के लिए ०.५ M NaOH और ०.५ -३ % हाइड्रोजन पेरोक्साइड के साथ सांद्रता किया गया था, जिसकी उपज ३४. ८ wt.% पाई गई थी जिसमें ०.५ wt.% की लिग्निन था।

Phragmites karka, जिसे सरकंडा के रूप में भी जाना जाता है, एक बारहमासी घास और एक अत्यधिक आक्रामक फसल प्रजाति है, जो देशी जैव विविधता और वनस्पति के साथ प्रतिस्पर्धा करके पारिस्थितिक समस्याएं पैदा करती है। इस अध्ययन में मोनोमेरिक शर्करा (monomeric sugars like glucose and xylose) का उत्पादन करने के लिए फ्राग्माइट्स का जल विश्लेषण शामिल है, जिसके बाद शर्करा युक्त हाइड्रोलाइज़ेट का फूरफूरल (furfural) और लेवुलिनिक एसिड (levulinic acid) में उत्प्रेरक (catalytic) रूपांतरण होता है। उप-क्रांतिक जल विश्लेषण केंद्रीय समग्र डिजाइन विधि द्वारा चर तापमान (१५०-२३० डिग्री सेल्सियस), प्रतिक्रिया समय (१५-६० मिनट) और फ्रीड एकाग्रता (२-५ wt.%) पर किया गया था।

तापमान बायोमास जल विश्लेषण को प्रभावित करने वाला सबसे प्रमुख कारक पाया गया। बायोमास जल विश्लेषण से कुल अपचायक शर्करा (TRS) की उपज २.१ - १८.१ % की सीमा में थी जहां उच्चतम उपज इष्टतम तापमान (१९० डिग्री सेल्सियस), प्रतिक्रिया समय (३७.५ मिनट) और फ़ीड सांद्रता (२ wt.%) पर प्राप्त की गई थी। Phragmites के सबक्रिटिकल वाटर विश्लेषण के दौरान, उच्च तापमान (२३० डिग्री सेल्सियस) और प्रतिक्रिया समय (३७.५ मिनट) पर प्राप्त दो मुख्य अवक्रमित वाले उत्पाद फुरफुरल (furfural) (८.२ wt.%) और 5-हाइड्रॉक्सीमिथाइलफुरफुरल (११.७ wt.%) थे। हालांकि, २३० डिग्री सेल्सियस और ६० मिनट की लंबी प्रतिक्रिया समय पर, 5-हाइड्रॉक्सीमिथाइलफुरफुरल की उपज कम होकर ५.१ % हो गई, जो कि ह्यूमिन (humins) में परिवर्तित हो गई, जबकि फुरफुरल (furfural) की उपज ९.९ wt.% तक बढ़ गई। ZrO_2 , TiO_2 , $Zr_{0.5}Ti_{0.5}O_2$, WO_3-ZrO_2 , WO_3-TiO_2 और $WO_3-Zr_{0.5}Ti_{0.5}O_2$ जैसे उत्प्रेरक Phragmites के उप-क्रांतिक वाटर (जल) विश्लेषण से प्राप्त शर्करा-समृद्ध हाइड्रोलाइज़ेट के रूपांतरण में शामिल थे। WO_3-ZrO_2 के साथ उच्चतम शर्करा रूपांतरण ९२ % पाया गया जिसके परिणामस्वरूप फ़्यूरफ़्यूरल (५१ %) और लेवुलिनिक एसिड (३४ %) की पैदावार हुई। विशेष उत्प्रेरक (जैसे, WO_3-ZrO_2 , WO_3-TiO_2 और $WO_3-Zr_{0.5}Ti_{0.5}O_2$) की गतिविधि लुईस (Lewis) और ब्रॉन्स्टेड (Bronsted) एसिड साइटों के सहक्रियात्मक प्रभावों पर निर्भर करती है। इसके अलावा, इस फ़ीडस्टॉक का उपयोग हरित (environmentally friendly) और एकीकृत (integrated) तकनीक का उपयोग करके सेल्यूलोज़ फाइबर को अलग करने के लिए भी किया गया था। सेल्यूलोज़ फाइबर को समेकित उप-क्रांतिक जल विश्लेषण (SbCW), पल्लिंग और ब्लीचिंग प्रक्रिया द्वारा वेटलैंड रीड ग्रास (*Phragmites karka*) से अलग किया गया था। SbCW उपचारित बायोमास को सेल्यूलोज़ फाइबर को अलग करने के लिए लुगदी (pulp) और विरंजन (bleaching) प्रक्रिया के माध्यम से किया गया था। उच्चतम सेल्यूलोज़ उपज ३५.१ wt.% पाई गई, जिसमें ०.४ wt.% की अवशिष्ट लिग्निन अंश ०.५ M NaOH पर प्राप्त किया गया था।

उप-क्रांतिक जल विश्लेषण को शामिल करने के साथ, सेल्यूलोज़ की एक उच्च उपज क्रमशः ६५.३ % और ६५ % के क्रिस्टलीयता सूचकांक (crystallinity index) के साथ *Phragmite karka* और *Cannabis indica* से प्राप्त की गई थी। प्रभावी उप-क्रांतिक जल विश्लेषण प्रक्रिया के कारण, बाद की प्रक्रियाओं जैसे लुगदी और विरंजन के लिए कम गंभीर परिस्थितियों की आवश्यकता होती है। इसके अलावा, एक तुलनात्मक अध्ययन में, अनुपचारित बायोमास, सबक्रिटिकल वाटर हाइड्रोलाइज़्ड बायोमास और सेल्यूलोज़ फाइबर को कई भौतिक रासायनिक लक्षण वर्णन उपकरण जैसे कि समीपस्थ, अंतिम और संरचना विश्लेषण, थर्मोग्रैविमेट्रिक विश्लेषण, फूरियर-ट्रांसफॉर्म इंफ्रारेड, फूरियर-ट्रांसफॉर्म नियर-इन्फ्रारेड और रमन की

विशेषता थी। स्पेक्ट्रोस्कोपी, एक्स-रे विवर्तन, ^{13}C सॉलिड-स्टेट न्यूक्लियर मैग्नेटिक रेजोनेंस स्पेक्ट्रोस्कोपी, स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी, ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी और एटॉमिक फोर्स माइक्रोस्कोपी तकनीकों का उपयोग क्रिस्टलीयता सूचकांक, कार्बन सामग्री, डिहाइड्रिफिकेशन, सेल्युलोज रिकवरी के साथ-साथ थर्मल स्थिरता (thermal stability) और आकारिकी का अनुमान लगाने के लिए किया गया था। सेल्युलोज फाइबर की। इन प्रक्रियाओं द्वारा, दोनों आक्रामक बायोमास का उपयोग किया गया और इन फीडस्टॉक्स के लिए एक नया शोध मंच खोला गया।

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LIST OF SYMBOLS

amu: atomic mass unit

CDCl_3 : chloroform-d

CV: co-efficient of variance

F: Feed concentration

H_3O^+ : Hydronium ion

HSO_3 : bisulfate

m/z: mass to charge ratio

MHz: mega hertz

N: normal

p-value: probability value

R^2 : co-efficient of determination or regression

R_{RMS} : root-mean-square roughness

T: Temperature

v/v: volume by volume

w/v: weight by volume

$\text{WO}_3\text{-TiO}_2$: Tungsten trioxide impregnated

$\text{WO}_3\text{-ZrO}_2$: Tungsten trioxide impregnated

wt.%: Weight percentage

$\text{Zr}_{0.5}\text{Ti}_{0.5}\text{O}_2$: Zirconium-titanium mixed oxide

ZrOCl_2 : Zirconyl chloride

LIST OF ABBREVIATION

5-HMF: 5-hydroxymethyl furfural	H-USY: Ultrastable Y in the protonic form
ABPR: Automatic Back Pressure Regulator	IC ₅₀ : Half-maximal inhibitory concentration
ADF: Acid Detergent Fibre	ICP-MS: Inductively coupled plasma-mass spectrometer
ADL: Acid Detergent Lignin	KIT: Korea Advanced Institute of Science and Technology
AFM: Atomic force microscope	MCM: Mobil Composition of Matter
ANOVA: Analysis of variance	MSU: Michigan State University
ASTM: American Society for Testing and Materials	NDF: Neutral Detergent Fibre
ATR: Attenuated total reflectance	NH ₃ -TPD: Ammonia temperature-programmed desorption
BHT: Butylated hydroxy toluene	NIR: Near Infrared
BOD: Biological Oxygen Demand	¹ H NMR: Proton Nuclear Magnetic Resonance
CBD: Cannabidiol	NREL: National Renewable Energy Laboratory
CBN: Cannabinol	SBA: Santa Barbara Amorphous
CCD: Complex Composite Design	SbCW: Subcritical water
COD: Chemical Oxygen Demand	SCCO ₂ : Supercritical CO ₂
COK: Centre for Research Chemistry and Catalysis	SEM: Scanning electron microscope
CrI: Crystallinity Index	TCD: Thermal conductivity detector
DNSA: 3,5-dinitrosalicylic acid	THC: Tetrahydrocannabinol
DPPH: 2,2-Diphenyl-1-picrylhydrazyl	THCA: Tetrahydrocannabinolic acid
DTG: Differential thermogravimetric analysis	THCV: Tetrahydrocannabivarin
EDTA: Ethylenediaminetetraacetic acid	TRS: Total reducing sugars
FTIR: Fourier-Transform Infrared Spectroscopy	UHMI: Ultra High Matrix Introduction
GC-MS: Gas chromatography- Mass spectrophotometry	ZSM: Zeolite Socony Mobil
HPLC: High-Performance Liquid Chromatography	