Valorization of Invasive Plant Species to Produce Bio-Chemicals and Bio-Products through Greener Approaches

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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 CO_2 ($SCCO_2$) 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 CO_2 ($SCCO_2$) extraction through central composite design (CCD) by taking CO_2 pressure, temperature and extraction time as the experimental parameters. Among these three parameters, CO_2 pressure had a greater impact on the extraction process than the other two factors out of all the variables. With a CO_2 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), ^{1}H NMR (Proton Nuclear Magnetic Resonance) spectroscopy, and GC-MS (Gas chromatography- Mass spectrophotometry). The extract contains four primary cannabinoids, including CBD (Cannabidiol), THCV (Tetrahydrocannabivarin), Δ^9-THC (Δ^9-Tetrahydrocannabinol), and Δ^8-THC (Δ^8-Tetrahydrocannabinol), as well as two distinctive terpenoids: cis-caryophyllene and α-humulene. The residue biomass generated from the $SCCO_2$ 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 IC_{50} (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 IC_{50} 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 ZrO_2, TiO_2, $Zr_{0.5}Ti_{0.5}O_2$, WO_3–ZrO_2, WO_3–TiO_2 and WO_3–$Zr_{0.5}Ti_{0.5}O_2$ 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 WO_3–ZrO_2 resulting in the yields of furfural (51%) and levulinic acid (34%). The activity of particular catalysts (e.g., WO_3–ZrO_2, WO_3–TiO_2 and WO_3–$Zr_{0.5}Ti_{0.5}O_2$) 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, ^{13}C 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.