Electronic Supplementary Material (ESI) for Green Chemistry.

Sustainable Preparation of Oxidized Graphitic Material from Wheat Straw Using a Deep Eutectic Solvent for Superactivity of Cellulase

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Supporting Information

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Annexure S1:

List of merits that our new method/technique for the dissolution of Wheat Straw and formation of oxidized graphitic material holds over the existing methods reported in literature.

S. No	Merits of our method	Description of Our Method	Description of Reported Methods
1.	Prevention (Less Waste)	**Our method:** For the dissolution and regeneration of wheat straw, lactic acid-based DES has been synthesized, which produces no waste as byproduct during its synthesis. Regeneration of dissolved wheat straw from DES is carried by using water as an antisolvent, which is again greener in nature, and the process don't produce any waste.	**Reported Methods**: Volatile Organic Solvents (VOSs) used for dissolution of biomass release toxic fumes in the environment and harms it.[1-2] There are many reported methods for the synthesis of graphitic material. The methods such as transition metals catalyzed chemical vapor deposition (CVD) process involves the usage of toxic metals which harms the environment. The another most commonly employed method *i.e.* liquid phase exfoliation process produces low quality graphitic material due to the presence of residual volatile solvents on the surface, which tend to be environmentally toxic.[3] In the pyrolysis method, high concentration of acid or alkali are used which again produces toxic fumes in the environment. [4-6]
2.	Atom Economy	Synthesis of DES falls in the category of "Atom economy" as the atoms involved in the process of synthesizing DES are incorporated in the product. Thus, minimizes the wastage at molecular level. Secondly, the atoms of components of wheat straw, which is dissolved in DES are also	Volatile Organic Solvents (VOSs) which are used for dissolution of biomass do produce waste at molecular level during their synthesis. Synthesizes of Ionic liquids (ILs) which are used for the dissolution of wheat straw, is a multi-step process which also involves purifications. Thus, there is high probability of atom economy loss.

		regained in regenerated material along with induction of new functional groups due to oxidation during the process of dissolution.	
3.	Less Hazardous Chemical Syntheses	Components of DES, the native wheat straw and water are chemically safe. Also, the synthesis of DES take place by simply mixing the components and heating them at optimum temperature, which makes the process less hazardous.	VOSs are used for dissolution of biomass and also used for synthesis of graphitic material are hazardous and toxic in nature. Besides this, ILs used for the pretreatment of biomass are often non-biodegradable in nature.[7] Also, the metals used in preparation of carbon-based material are also toxic in nature.[3,5]
4.	Design for Energy Efficiency (lower energy inputs, temp and pressure)	The DES has been synthesized at optimum temperature conditions (80 ^{o}C for 8 hours) and later the dissolution of wheat straw carried under microwave irradiations (30 mins and 60 ^{o}C) minimizes the energy consumption. Also, the dissolution take place at atmospheric pressure. At the last, regeneration step is also carried out at room temperature using water, which is a greener solvent.	Pre-treatment of biomass is usually carried out at high temperature and pressure conditions.[1] Also, many of the processes used to prepare carbon-based material take place at high temperature (300-1000 ^{o}C) conditions.[6]
5.	Safer Solvents and Auxiliaries	Components of DES are cheaper, easily available and biodegradable in nature. Dissolution of biomass is also carried out under ambient temperature condition which adds to safety. Water, which is a regenerating agent is also greener and safe.	VOS's used for dissolution of biopolymers are toxic and volatile. Many of the reported ILs used for the pretreatment of biomass are also toxic. Also, the solvents such as surface-active ionic liquids (SAILs) used in exfoliating process are costly and difficult to prepare in comparison to

		Thus, both DES and water, which are used in the whole process are safe, biodegradable and environmentally friendly.	DESs.[8-9]
6	Inherently Safer Chemistry for Accident Prevention (Ease in preparation)	Components of DES are safe and biodegradable. Synthesis take place by simply mixing the two components and heating them at 80 ^{o}C for 8 hours. Therefore, there are no chances of accident. Further, no VOS has been used during preparation of DES, which eliminates the risk of flammability during the reaction. Dissolution process is carried at 60 ^{o}C for 30 mins and under microwave irradiation thus optimum amount of heat is required to carry out the process. Moreover, the regeneration step which is another safer step, involves only the addition of greener solvent (water) at room temperature. Thus, the whole process *i.e.* formation of DES, dissolution of wheat straw, regeneration and application of regenerated material is safer and economic to be performed easily.	Solvents used in treatments and dissolution of biomass are difficult to prepare and handle.[10] Also, the solvents used in Hummer's method for exfoliation of graphene are not much safe and economic. Recently ILs which are used in exfoliating process are costly and moreover, their synthesis involves multiple number of steps and therefore, can't be prepared easily in comparison to DES.[8-10]
7.	Design for Degradation (Biodegradability)	Components used in preparation of DES are biodegradable in nature.	VOSs and ILs used in pretreatments and dissolution of biomass are non-biodegradable in nature.[8]

		Water used in regeneration process is also green and environment friendly. The recycling of DESs would limit the wastage and its environmental leakage.	Also, the solvents used in synthesis of carbon-based material persists in environment.
8.	Recyclability	DES has been recycled by simply evaporating water, which is another advantage. Further this recycled DES is used for the dissolution of wheat straw. Also, the regenerated material has been recycled many times for hosting enzymatic activity.	Solvents used in pyrolysis and CVD methods can't be recycled and reused again. Moreover, the solvents such as VOSs, SAIL, ILs and surfactants used in pretreatments and dissolution of biomass are used once and can't be recycled.
9.	Reuse	We reused the recycled DES and also the formed oxidized graphitic material without effecting its catalytic efficiency.	The solvents used in dissolution of biomass and formation of carbon-based material and studying the catalytic activity can't be used again.

Annexure S2:

Circular dichroism (CD) spectroscopic measurements have been carried out to probe the possibility of alterations in the 2° structure of Cellulase, which could favour the enzyme activity. The negative CD bands at 212 and 222 nm are peculiar of proteins having α-helices as dominant components of 2° structure.[12] However, a variety of Cellulases originating from different micro-organisms shows different CD spectra despite having *α*-helices as major components. The CD spectra of Cellulase under investigation exhibited a broad negative CD band centred at 212 nm without displaying any clear band around 222 nm, which supports the prevalence of α-helices as a major component and is in corroboration with literature reports.[13] Cellulase is a large single-domain α/β protein having a central β-barrel structure surrounded by a large number of α-helices. The central β-barrel comprises of 7 parallel β-strands where 6 of these strands are interconnected by α-helices and two of the loops at the COO-terminal of the barrel are widespread and stabilized by one di-sulphide bridge each. The interconnected helices along with the side chains from the extensive loops and barrel make the barrel a perfectly enclosed structure. A marginal decrease in the ellipticity without visible alterations in the shape of CD spectra upon the adsorption of Cellulase at graphitic material suggests marginal loss of 2° structure.

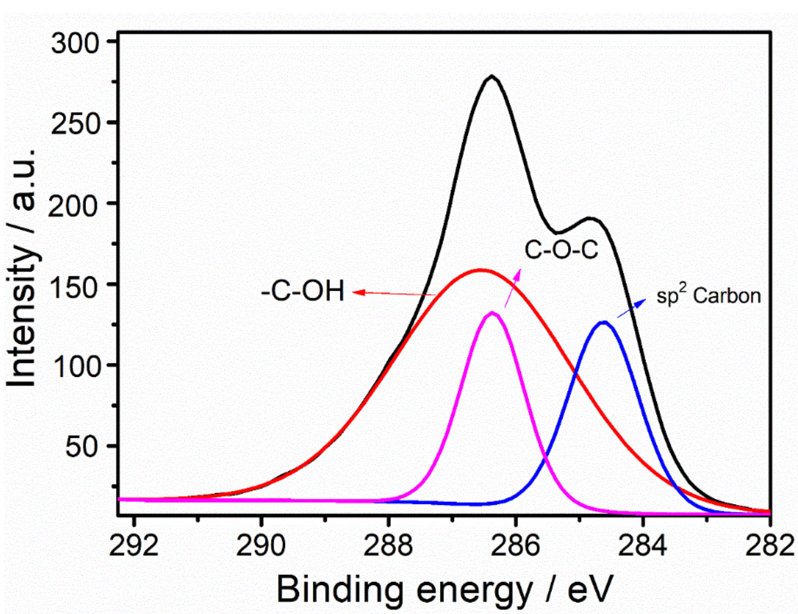

Figure S1: XPS spectra of native wheat straw.

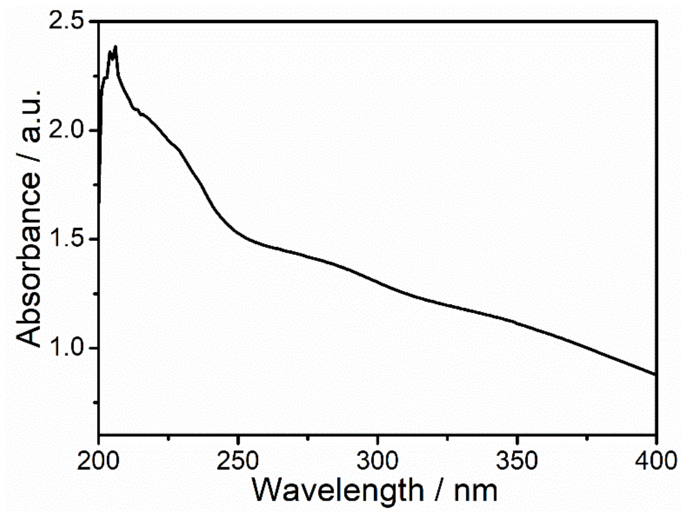

Figure S2: UV-Vis spectra of regenerated material in ethanol.

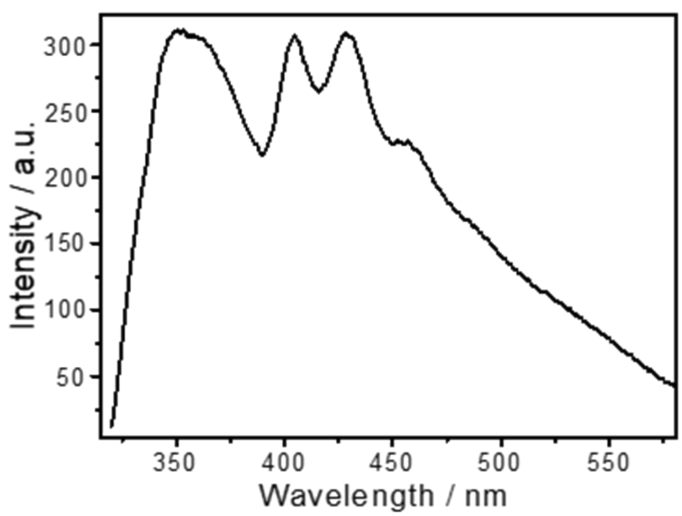

Figure S3: Fluorescence spectra of regenerated material in ethanol at λ_{ex}= 310 nm.

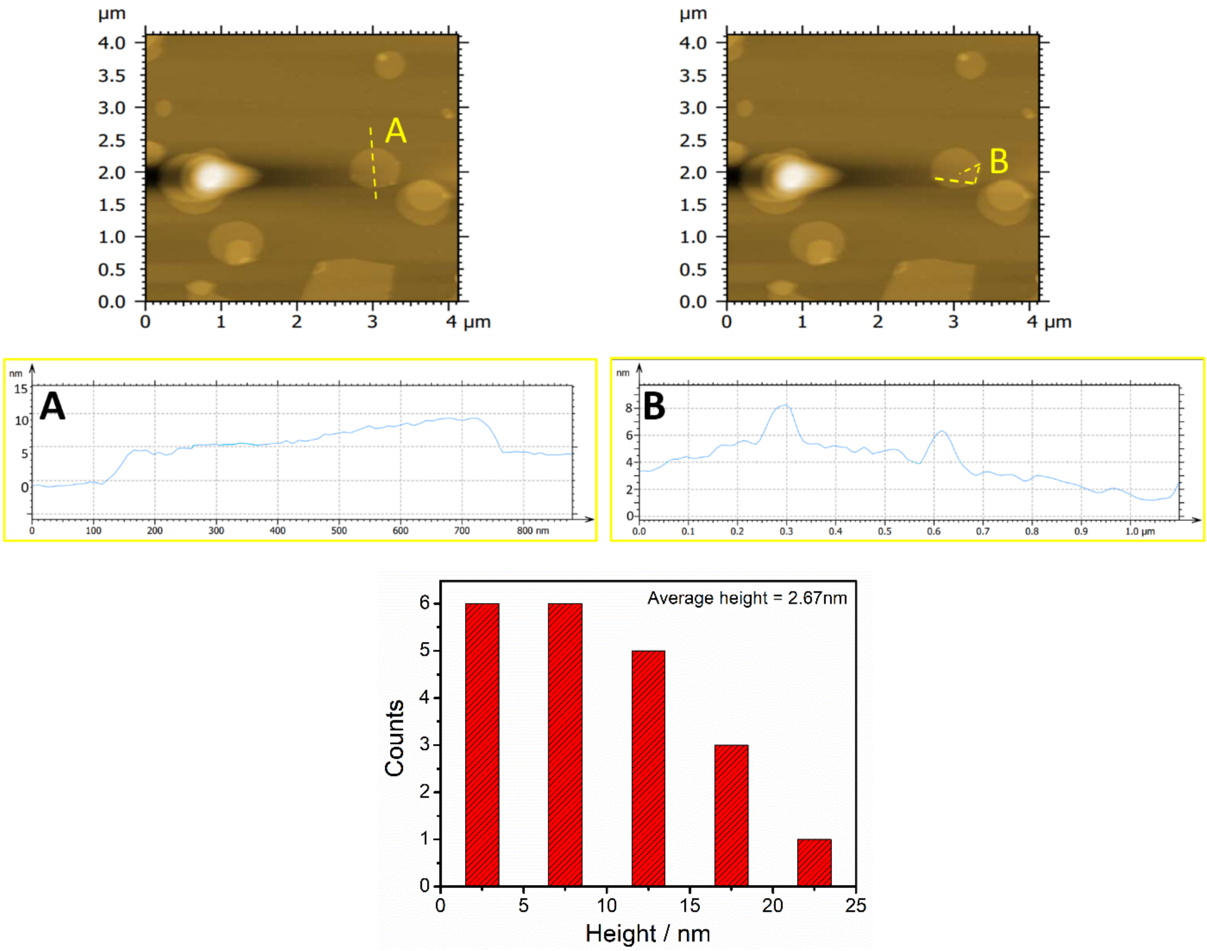

Figure S4: AFM images of regenerated material displaying the presence of sheets and particles and their respective height profiles.

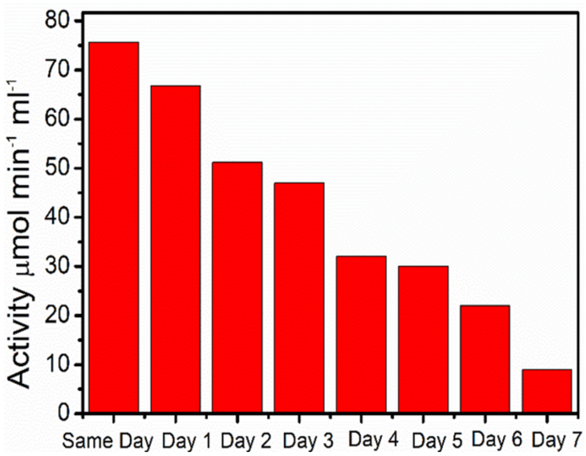

Figure S5: Activity of cellulase on recycled oxidized graphitic material at pH=7.

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