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Valorization of sugarcane straw to produce highly conductive bacterial cellulose / graphene nanocomposite films through *in situ* fermentation: kinetic analysis and property evaluation

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Abstract

Bacterial cellulose based nanocomposites have found a growing interest in recent decades due to their impressive inherent characteristics with potential applications in diverse sectors. However, there remain several challenges due to increased production cost, lower yield, and sustainability or biocompatibility issues after chemical-based modifications. This study demonstrates the fabrication of bacterial cellulose-reduced graphene oxide films via *in-situ* fermentation approach using abundantly available agricultural waste (sugarcane straw) as a feedstock. The presence of reduced graphene oxide at different concentrations in culture media, significantly altered the fermentation kinetics, as evident from kinetic parameter and yield coefficients. Higher yields of bacterial cellulose-reduced graphene oxide nanocomposites, with presence of strongly integrated network-like structures between bacterial cellulose nanofibers and reduced graphene oxide nanosheets were observed at 2 wt. % reduced graphene oxide loadings. Formation of such percolated networks was confirmed from improved mechanical properties and enhanced electrical conductivity, through both experimental and modeling investigations. The proposed *in-situ* fermentation technique to produce highly conductive bacterial cellulose-reduced graphene oxide films provides an alternative approach to meet the growing demands of biomass-derived renewable and sustainable biomaterials with commercial significance.

Keywords: Bacterial Cellulose; *In situ* fermentation; Fermentation Kinetics; Structural Properties; Electrical Conductivity.

1. Introduction

Bacterial cellulose (BC) are the exopolysaccharide products of the microbial fermentation which forms a thick white leathery pellicle at air-liquid interface under static culture conditions. Although the cellulose produced from bacteria and plant are chemically similar in nature, their assemblies and crystal structures show wide variation. BC is produced as ultra-thin nanofibers, which undergoes a network-like structure formation to yield high mechanical strength, crystallinity, water-absorption capacity and degree of polymerization. BC in its unmodified forms have been applied extensively in biomedical applications such as wound dressing, in the regeneration of skin tissues or burn treatments, and as substitutes to skin (Dugan, et al. 2013). Moreover, its applications

have been widened through strategic chemical modifications to induce antioxidant and antimicrobial properties. BC membranes have also been used for stimuli-responsive delivery of drugs, enzymes or biomolecules studied under both *in-vitro* and *in-vivo* conditions. Due to their high hydroxyl functionality, BC can be modified with a wide variety of metallic/inorganic nanoparticles or blending with conductive polymers for their potential applications as catalysts, bio-sensing, and electronic devices.

Although BCs are biodegradable, renewable and biocompatible their inherent properties such as low strength, stiffness and high fragility under hydrophilic conditions make them poor biomaterial for applications as commercial products. The variability in BC conversion, inhibitory effect of media components that eventually lowers the crystallinity of BC pellicles and reduced product yield are some of the operational challenges in BC production. BC based nanocomposites formation through sustainable routes with improved properties at large scale suitable for practical and commodity applications are addressed in this study.

BC modifications to fabricate the composites with improved properties can be done either through *ex-situ* chemical treatments or with *in-situ* bio-treatments. The most widely used methods for *ex-situ* modification have been through carboxylation, amidation, acetylation (Stumpf et al. 2018) etc. followed by incorporation of nanomaterials to prepare BC based nanocomposites with improved properties. The *ex-situ* BC modifications although been extensively studied; it generally involves hazardous chemicals which have issues related to environmental toxicity and biocompatibility. Moreover, the *ex-situ* modification encounters many challenges due to additional processing steps, such as energy-intensive disintegration process or BC solubilization in organic solvents/ionic liquids (Schlufter et al. 2006). Interestingly, *in-situ* BC modifications alleviate the aforesaid problems and opens up new research area for novel applications.

The *in-situ* BC nanocomposite fabrication are mostly carried out by using fermentation additives with carbon-based nanomaterials such as carbon nanotubes, graphene nanosheets (Nandgaonkar et al., 2014) or with inorganic nanomaterials such as tin oxide and conductive polymers such as polypyrrole and polyaniline (Liu et al. 2015). Recently *in situ* fermentation of BC with graphene/reduced graphene oxide/polyaniline and carbon nanofibers have been extensively studied by Wan et al. and co-workers through a novel layer-by-layer based self-assembly routes (Luo et al. 2019). Introduction of graphene dispersion in the inter-layers of BC was carried out through repetitive spraying method, which results in the formation of mechanically strong and thick three dimensional hydrogels (Luo et al. 2018a). Morphological and Raman spectroscopy studies confirm the presence of few-layered graphene nanosheets, with improved hydrogen bonding based molecular interaction (Luo et al. 2018b). Introduction of polyaniline results in the formation of free-standing BC based electrodes with excellent electrochemical properties with high charge storage capacity (Luo et al. 2018c). Interestingly, the BC/graphene nanocomposites developed through fermentation technique showed a high capacitance of ~215F/g, which are stable for almost ~5000cycles (Luo et al. 2018d). These studies were mainly focused on the development of BC based sustainable nanocomposites with improved properties and their potential applications in various sectors *viz.* electrodes/supercapacitors (Wan et al. 2018), adsorbents (Luo et al. 2018e), and scaffolds for tissue regeneration (Jin et al. 2016). Interestingly, strategic incorporation of graphene through sustainable routes of *in-situ* fermentation results in fabrication of composites with properties comparable to chemical based *ex-situ* modifications (Tan et al. 2018). The electrochemical properties of chemically modified free-standing graphene/polyaniline films (~286 F/g) (Salunkhe et al. 2014a) is comparable to the *in situ* fermented BC/graphene films (~215 F/g).

Moreover, the cyclic stability of bioprocessed films (upto~ 5000 cycles) were significantly higher than nanocomposite prepared through chemical routes (94% stability upto~2000 cycles) (Salunkhe et al. 2014b). It should be noted that the presence of such carbon-based nanomaterials as fermentation additives in fermentation media are expected to alter the microbial growth kinetics and process behavior, which plays a crucial role during up-scaled operations. One of the aims of present study is to understand the effect of RGO contents on the microbial growth dynamics, as determined through the bioprocessing kinetic parameters and yield coefficient values.

Many *in-situ* BC fabrication studies use glucose as a carbon source. However, sustainable process development approach encourages to explore the other biomass (such as lignocellulosic) for BC production in a cost-effective manner. Taking into consideration the economics and environmental concerns; use of inexpensive feedstocks such as agricultural or industrial waste products are highly desirable. To the best of authors' knowledge, utilization of waste agriculture harvests for production of BC based reduced graphene oxide (RGO) nanocomposites, through *in-situ* fermentation route with enhanced conductivity and structural properties, have not been reported till date.

The utilization of lignocellulosic biomass via bio-refinery strategies for co-generation of second or third generation biofuels, high-performance biomaterials, and variety of value-added biochemicals, provides a sustainable and renewable route to minimize the use of fossil materials. Traditional routes of disposal (burning or land-filling) of the large volume of wastes generated from agricultural farm's post-harvest are known to have severe environmental effects. Countries like Brazil and India are leaders in sugarcane production (dos Santos Rocha et al. 2017) and thereby generates surplus waste in the form of sugarcane bagasse and straw. It is estimated that processing of ~1,000 kg sugarcane produces almost 176 kg residues along with 231 kg bagasse on the dry basis. The sugarcane farming in Brazil sums up to produce ~93.3 million tons of waste sugarcane straws every year. Unfortunately, the sugarcane waste management after harvest is poorly addressed with huge biomass being subjected to burning, which has both health as well as environmental threats. Although, sugarcane bagasse has been utilized recently, to produce bio-ethanol, bio-butanol or for bioenergy in power generation, the waste generated from straw was rarely been valorized. Sugarcane straw contains significant fractions of cellulose (35-45%), hemicellulose (25-35%), lignin (10-25%) and other minor inorganic compounds. These components can be utilized to produce value-added biochemicals by using several pretreatment techniques such as acid hydrolysis, enzymatic hydrolysis or hydrothermal treatment. Interestingly, BC yields from biomass are generally higher than from pure glucose as carbon source.

BC production by utilizing agricultural sources *viz.* coffee cherry husk, rice bark, wood sugars, corn steep liquor have already been studied (Costa et al. 2017). Additionally, industrial byproducts such as waste generated from beer processing, textile or wastewater from chocolate process industries have also been explored to produce BC (Li et al., 2015). Although sugarcane molasses has been utilized to produce BC, the sugarcane straw have been seldom used to produce BC.

The current study utilizes the waste sugarcane straw hydrolysates to produce BC/RGO nanocomposites for its high-value biomedical applications. The pretreated sugarcane straw hydrolysate was exploited in presence of RGO by using *Komagataeibacter xylinus*, to study the fermentation behavior. The present study also evaluated the formation of percolated network-like structure for BC/RGO nanocomposites through micro-mechanical or continuum models, which tremendously improved the conductivity and structural properties. Interestingly, the presence of a

hierarchical assembly of BC nanofibers in inter-layers of RGO (at lower loadings) improved the tensile stress, toughness as well as elongation behavior simultaneously, which otherwise is difficult to achieve. Addition of RGO to culture media, though chemically inert, was found to significantly affect the microbial growth kinetics, depending on its concentration. The *in-situ* BC/RGO nanocomposites are expected to tailor and substantially improve the physicochemical properties. This study provides a sustainable route, which upconverts waste into value-added bio-nanocomposites with the promising application as commodity products.

2. Materials and Methods

The microbial strain *Komagataeibacter xylinus* ATCC 11142, used for BC fermentation, was purchased from Leibniz Institute DSMZ-German Collection of Microorganism and Cell Culture, Germany. The freeze-dried microbial culture was revived as per DSMZ protocol. Sugarcane straw used in this work was kindly provided by Ipiranga Agroindustrial S.A. mill (Descalvado, SP, Brazil). The biomass was dried at room temperature until 10% moisture content. Afterwards, it was milled in a Willey type mill (model SP-30, SPLABOR, Presidente Prudente, SP, Brazil) to a particle size of 10 mesh (2 mm), stored into plastic bags and kept in a freezer (-8 °C) to avoid contamination prior to pretreatment. The commercial enzymatic complex used in the hydrolysis experiments was Cellic®CTec2 (Novozymes Bioag A/S, Denmark) with a filter paper activity of 240 FPU/mL (Ghose, 1987). All the chemicals and nutritional media components used in this study were of Analytical grade and purchased from Sigma Aldrich, Finland Oy. Expandable graphite (EG, 99.9%) used in this study was purchased from Asbury Carbons (USA) and was modified as discussed in subsequent section.

2.1 Pretreatment of sugarcane straw

The liquid hot water (LHW) or hydrothermal pretreatment was performed in a 5.5 L stainless steel reactor (model 4584, Parr Instruments Company, Moline, IL, USA) equipped with propeller stirrer, heater, and temperature controller. A quantity of 200 g of dry and milled (2 mm particle size) sugarcane straw was mixed with distilled water (ratio 1:10 w/v) and set inside the reactor. The system was heated up to 195 °C, agitated at 200 rpm, and maintained for 10 min. The reactor was then cooled down to 50 °C to separate solid and liquid fractions by using filtration. Finally, the solid fraction was washed with water to remove solubilized contents, until neutral pH was reached (Batista et al. 2019). The pretreated sugarcane straw sample was further hydrolyzed to release monomeric sugars.

The pretreated biomass was characterized to analyze its chemical composition, according to National Renewable Energy Laboratory's (NREL) procedure (Sluiter et al., 2008).

2.2 Enzymatic hydrolysis of pretreated sugarcane straw samples

Enzymatic hydrolysis experiments were carried out by using 10 % (w/v) biomass loading and 15 FPU/g_{cellulose} of enzyme dosage. These conditions were defined in order to reach the values of cellulose-to-glucose conversions above 80 % in 48 h enzymatic hydrolysis. The hydrolysis experiments were performed in 200-mL Erlenmeyer flasks containing 100 mL reaction medium at pH 5.0 (50 mM citrate buffer) agitated in incubator shaker (Certomat® HK, Germany) at 250 rpm and 50 °C. The liquid hydrolysate was separated from solid slurry by centrifugation (9,600 g, 20 min, 4 °C) for further use. Individual and total sugar analysis was done by using high-performance liquid chromatography (Waters Alliance e2695), equipped with a refractive index detector (Waters

2414) and column ionic exclusion (Rezex ROA-Organic acid H+ (8%)) using 5 mM H₂SO₄ at 0.6 mL/min and 65 °C.

Cellulose-to-glucose conversion (X_{C-G}) and hemicellulose-to-xylose conversion (X_{X-G}) were determined according to Eq. (1) and (2),

$$X_{C-G}(\%) = \frac{[Glucose]}{[Biomass] \times f_C \times 1.11} \cdot 100 \quad (1)$$

where [Glucose] is the glucose concentration (g/L) released at the end of the enzymatic hydrolysis. The term “[Biomass] × f_C × 1.11” corresponds to theoretical glucose concentration, where [Biomass] is concentration (g/L) of dry pretreated sugarcane straw added at the beginning of hydrolysis process; f_C is the cellulose fraction of pretreated sugarcane straw (0.548 g/g - as detailed in results and discussion section); 1.11 is the stoichiometric factor of cellulose to equivalent glucose.

$$X_{X-G}(\%) = \frac{[Xylose]}{[Biomass] \times f_H \times 1.14} \cdot 100 \quad (2)$$

where [Xylose] is the xylose concentration (g/L) released at the end of enzymatic hydrolysis; f_H is hemicellulose fraction of the pretreated sugarcane straw (0.082 g/g - as detailed in results and discussion section); 1.14 is the stoichiometric factor of hemicellulose to equivalent xylose.

2.3 Fabrication of Reduced Graphene Oxide (RGO)

Expandable graphite (EG, 99.9%), procured from Asbury Carbons (USA) (Product Number: 3772) was subjected to thermal exfoliation in a muffle furnace followed by surface modification, as reported in our previously study (Dhar et al. 2018). The partial oxidation of exfoliated graphene (500 mg) (dispersed in methanol) was carried out by using sonication (at an amplitude of 30%) in presence of hydrogen peroxide (2wt. %) for 30 mins. Oxidized graphene suspension dispersed in methanol was centrifuged and washed repeatedly to remove trace chemicals. Thereafter, reduced graphene oxide suspension was dried in hot air oven to obtain dried powdered which hereafter represented as RGO.

2.4 Inoculum preparation for BC fermentation

Pre-inoculum of *K. xylinus* was prepared in 100 mL of sterile Hestrin- Schramm (HS) media (g/L): glucose (20.0), yeast extract (5.0), peptone (5.0), disodium hydrogen phosphate (2.5) and citric acid monohydrate (1.15) at pH 6.5, 30 ± 1°C and 100± 2 rpm for 24 hours. The OD₆₀₀ was measured throughout cultivation for consistent microbial growth. This 10% v/v inoculum was used to produce BC in presence of different RGO concentrations.

2.5 Production of BC/RGO composites using pretreated sugarcane cane hydrolysate

Low-cost agricultural residue such as sugarcane straw was utilized in this study to produce BC/RGO nanocomposite films by using *K. xylinus* fermentation. The glucose concentration obtained after enzymatic hydrolysis (with an initial concentration of ~54.2%) was adjusted to ~20 g/L. Other nutrients such as yeast extract (5.0 g/L), peptone (5.0 g/L), disodium hydrogen phosphate (2.5 g/L) and citric acid monohydrate (1.15 g/L) were added to the hydrolysates, at pH

6.5. RGO at different media concentration of 1, 2, 3 and 5 wt. % was added after which it was subjected to sterilization in an autoclave. The BC/RGO fermentation was carried out after 10% pre-culture inoculation under the static condition at 30°C for 15 days.

The microbial kinetic study was also performed to investigate the effect of partially oxidized RGO (1 - 5 wt. %) on BC yields. The samples (replicative flasks of 20 mL) were collected after time intervals of 1,3,5,6,7,9,10,11,12 and 15 days to analyze the microbial growth as well as BC production yield. Control experiments were performed simultaneously without addition of RGO during BC fermentation. All the experiments were performed at least in triplicates and results mentioned are an average $\pm$ standard deviation.

2.6 Purification and quantification of BC/RGO nanocomposites

BC/RGO nanocomposite membranes produced at the air-water interface during fermentation after 15 days was separated and pressed to remove excessive aqueous media fractions. The membrane was subjected to chemical treatment with 0.1 M sodium hydroxide at 80°C for 90 minutes to remove any attached cells and other impurities. These membranes were subsequently washed with excess water repeatedly until it reached pH 7 and stored at 4°C. The yield of BC/RGO nanocomposite films were calculated by measuring the dry weight and taking into consideration the volume of medium used for fermentation. BC membranes were dried in a hot air oven for 3 days at 40°C.

2.7 Determination of biomass and sugar concentration

Sugar concentration after enzymatic hydrolysis and after fermentation experiments were analyzed using high-performance liquid chromatography (HPLC) (Shimadzu HPLC. LC 20AD series) attached with an Agilent Hi-Plex H column along with guard column maintained at 65°C. The quantitative analysis was done by using a differential refractometer detector. HPLC was equipped with auto-sampler and injection volume of 20 μ L with sulphuric acid (5mM) as eluent at 0.6mL/min. The HPLC system was pre-calibrated with standard solutions of sugars and concentration of each analyte measured by considering anhydrous mass correction and dilution factors. The microbial growth with respect to time was measured by optical density (OD₆₀₀) analysis using UV-Vis spectrophotometer (Perkin Elmer, Lambda 35). The microbial cells trapped within BC pellets, were evaluated by hydrolyzing them using cellulases (50°C for 1 hour) followed by filtration and dry weight determination.

3. Results and Discussions

The present study utilizes low-cost agriculture wastes (sugarcane straw) for the production of highly conductive BC/RGO nanocomposite films via *in-situ* fermentation approach, as illustrated in Figure 1. Enzymatic hydrolysis of sugarcane biomass using endoglucanases under optimized conditions resulted in the formation of sugar derivatives, which were utilized as a carbon source during BC fermentation. The bioprocessing kinetic parameters for BC/RGO fermentation were evaluated followed by investigations of physio-chemical, structural and electrical properties which are discussed in subsequent sections.

3.1 Pretreatment and enzymatic hydrolysis of sugarcane straw

The chemical compositions of sugarcane straw before and after pretreatment are shown in Table 1. The LHW pretreatment of sugarcane straw resulted in the loosening of lignin and reduced

crystallinity of biomass making enzymes easily accessible for the conversion of lignocellulosic components to fermentable sugars.

After 48 h, the enzymatic hydrolysis released the following fractions: 49.2 g/L of glucose; 6.1 g/L of xylose; 1.8 g/L of cellobiose and 2.9 g/L of acetic acid. The conversion of cellulose-to-glucose was found to be 81 %, which indicate that cellulose exploitation was satisfactory during the short time of hydrolysis reaction.

Table 1: Chemical composition of sugarcane straw before and after LHW pretreatment

Component (%)	Before pretreatment*	After pretreatment*
Cellulose	34.23 ± 0.35	54.77 ± 0.30
Hemicellulose	23.21 ± 0.31	8.22 ± 0.08
Lignin	24.11 ± 0.52	26.70 ± 0.99
Ash	7.12 ± 0.18	6.67 ± 0.07
Protein	2.68 ± 0.03	2.27 ± 0.05
Extracts	8.31 ± 0.41	-
Total (%)	99.57 ± 0.83	98.63 ± 1.04
Mass yield (R_M) of LHW (%) ^a	-	50.52 ± 2.13

^a The mass yield was calculated as $R_M (\%) = m_{\text{final}}/m_{\text{initial}} \cdot 100$, where m_{final} (g) is the amount of dry biomass after pretreatment and m_{initial} (g) is the amount of dry biomass before pretreatment.

* Values reported are average ± standard deviation of three replications

Figure 1: Schematic representation of overall BC/RGO nanocomposite film formation by using sugarcane straw hydrolysates

3.2 In-situ BC/RGO nanocomposite production by using *K. xylinus*

The *in situ* fermentation approach for production of the BC/RGO nanocomposites using the waste sugarcane straws as carbon source was carried out with functionalized RGO. The functionalization of RGO (partially oxidized) was carried out through mild peroxide treatments (Dhar et al. 2018) and then added to culture media at different concentrations (from 1 to 5wt. %). The partial oxidation in presence of peroxides resulted in incorporation of hydroxyl and epoxy groups on RGO. Additionally, the presence of complex carbohydrates and proteins present in culture media helped in improving the dispersion and stabilization of RGO (Nandgaonkar et al. 2014). Interestingly, it was observed that RGO undergoes gradual reduction (in presence of media components) during fermentation process with formation of thick BC/RGO hydrogels at air-water interface (Figure 1). However, it should be noted that the high hydroxyl functional groups present in BC (produced during *in situ* fermentation) creates a reducing environment, which is expected to have significant effect on reduction of RGO (Zhang et al. 2010). This resulted in improved dispersion of RGO nano-flakes in between BC nanofibers, forming a percolated network-like structure, leading to enhanced electrical conductivity and structural behavior of fabricated films (Figure 1), as discussed in the subsequent sections. The growth kinetics of *in-situ* BC/RGO fermentation process was assessed to study the effect of RGO on microbial growth kinetics and its correlation with physicochemical properties of BC/RGO films (discussed in the subsequent section). The BC/RGO films formed after *in situ* fermentation are light, highly flexible and easy to handle, as also evident from the high tensile strength and toughness (discussed in section 3.6). The current study provides a novel and greener approach to develop high value composite material by using low cost agricultural waste envisioning it's better performance and commercial viability.

Figure 2: Influence RGO concentration on (a) substrate consumption (g/L) (b) Biomass formation (g/L) (c) BC/RGO film formation (g/L) and (d) growth rate and yield coefficients, by using of *K. xylinus* during static *in-situ* fermentation at pH 6.5, 30°C and utilizing sugarcane straw hydrolysate.

Table 2: Kinetic parameters and yield coefficients determined for *in-situ* BC/RGO formation at varying RGO concentrations under static conditions by using *K. xylinus* at pH 6.5, 30°C and utilizing sugarcane straw hydrolysate.

Process Parameters	BC	BC-1RGO	BC-2RGO	BC-3RGO	BC-5RGO
Bacterial Cellulose Production					
Cellulose (g/L)	2.96	4.10	4.51	2.25	2.10
Q_p (g/L/day)	0.075	0.09	0.10	0.08	0.09
$Y_{P/S}$ (g/g)	0.119	0.164	0.180	0.096	0.098
$Y_{P/X}$ (g/g)	1.02	1.48	1.42	1.12	1.10
$Y_{P/X}$ (g/g) (in presence of glucose)	0.460	0.449	0.492	0.50	0.605
Substrate Consumption					
Glucose Consumed (%)	99.7	99.7	99.8	93.6	85.6
Q_s (g/L/day)	0.526	0.528	0.562	0.910	0.791
Cell Growth parameters					
$Y_{X/S}$ (g/g)	0.116	0.110	0.126	0.085	0.088
Cell (g/L)	2.9	2.82	3.20	2.02	1.92
μ_{max} (/day)	0.69	0.77	0.96	0.78	0.72
K_s (g/L)	0.08	0.16	8.1	2.8	3.0

3.3 Microbial growth kinetics study during *in-situ* BC/RGO nanocomposite formation

The utilization of waste agricultural residues for the production of highly conductive BC/RGO nanocomposite films require a precise knowledge of process parameters and microbial growth kinetics that provides an unique approach to evaluate its scalability. The effect of RGO nanofiller content on microbial growth performance was evaluated. The functionalized RGO when dispersed with sugarcane straw hydrolysate formed a stable black suspension. The microbial culture shows a stagnant increase in cell concentration for initial 2-4 days, suggesting a lag phase. The substrate consumption was still significantly higher in culture containing 1wt. % RGO. The BC pellicle formation was initiated at air-water interface after 2 days of fermentation. Interestingly, the culture color after 2 days was gradually transformed to pale yellow with growing BC pellicles. Fermentation yield of BC/RGO films was significantly increased (in presence of RGO upto a threshold concentration of ~2wt. %), especially due to improved interfacial interaction of RGO with BC fibers in presence of sugarcane hydrolysates with high C/N ratio. Microorganisms in exponential phase showed rapid substrate consumption with higher BC/RGO yields at various RGO concentrations. However, the specific growth rate and yield coefficients were significantly affected by different RGO concentrations in culture media. The maximum specific growth rate was highest (μ_{max} 0.96/day) when 2 wt.% RGO was used, suggesting that RGO at lower concentrations supported to improve BC/RGO yield by two-fold (Fig. 2d). Moreover, BC/RGO hydrogel formed at 2 wt.% RGO, was also thicker and denser than other experiments. The black colored RGO nanoflakes were dispersed in between BC layers. This is possibly due to negative

surface charge and hydrophobic interaction from RGO which acts as an anchoring site for BC adhesion, which favorably enhances the microbial growth rate and BC yields. Microbial exponential growth phase was predominant until 12 days of fermentation for all RGO concentrations tested, with gradual declaration afterward. It was observed that the percent glucose consumption was significantly higher when 1 to 3 wt.% RGO was introduced, with almost complete substrate utilization after 10 days of fermentation. Interestingly, the BC/RGO film formation was continued until 14-15 days of fermentation. It could be inferred that the *in-situ* BC/RGO fermentation followed mixed growth associated product formation profile for RGO concentrations until 3 wt.%. RGO content >3wt.% showed high fractions of unconsumed glucose (~15%) in culture media with almost constant BC production rate after 10 days, suggesting the inhibitory effect of RGO at higher concentrations. At higher RGO content of ~3 and 5 wt. %, the yield of BC/RGO films relatively decreased possibly due to lower consumption of glucose and reduced specific growth rate ($\mu_{\max}$ ~0.71/day). The growth rate of culture was eventually declined during microbial stationary phase (12-14 days). However, BC production was still high (with 1-3wt.% RGO) even in stationary phase. Moreover, in comparison to pure glucose as sole carbon source, the production of BC and its nanocomposites were significantly high due to the presence of complex mixtures of sugars and acetic acid in sugarcane hydrolysates. It could be concluded that the waste sugarcane straw residues can be successfully used as a feedstock for BC/RGO production, in presence of lower RGO concentrations (2wt. %) without significantly affecting microbial growth behavior.

Table 2 evaluates the bacterial kinetics parameters and their behavior in presence of different RGO concentrations. The BC production by utilizing sugarcane hydrolysates was improved when compared with BC production by using pure glucose, from 1.06 g/L (unpublished data Table 1) to 2.96 g/L. BC/RGO production was further improved (4.51 g/L) in presence of RGO (at 2wt. %) with improved yield, production rate and overall productivity (Table 2). The bacterial kinetic parameters and thereby BC/RGO production rate was significantly affected in presence of higher RGO concentrations (>4wt. %). Our previous study (unpublished data, Supplementary Fig.1) showed that RGO addition at 5wt. % RGO significantly decreased the BC production to 0.72 g/L when pure glucose was used as a substrate. However, current study utilizes sugarcane hydrolysates in presence of 5wt.% of RGO to produce considerably higher BC up to 2.10g/L. This increased BC production is probably due to the presence of xylose sugars and acetic acid derivatives present in sugarcane straw hydrolysate, which facilitated the improved microbial growth. Xylose and acetic acid have shown to boost BC production with improved physicochemical properties (Yang et al., 2014; Ishihara et al., 2002). Interestingly, the lignin residues present in hydrolysates are generally not metabolized. However, its presence in BC fibers helps in improving structural integrity (as discussed in subsequent section). Presence of lignin in trace quantities was also embedded in BC nanofibers that mimics the plant-derived cellulose composition. Further, negatively charged lignin in presence of RGO is a good substrate for the adherence and growth of BC fibers. The utilization of lignocellulosic biomass has a favorable effect on BC production and its later modification. Additionally, lignocellulosic biomass also reduced the overall bioprocess cost. Therefore, kinetic parameters evaluation suggests that *in-situ* BC/RGO fermentation largely depends on substrate type and RGO concentration with improved physicochemical and structural properties as discussed in subsequent sections.

3.4 Investigating inherent properties of BC/RGO films: Effect of fermentation conditions

The crystallinity and crystal structure were evaluated to study the effect of RGO on formation of BC fibers during *in-situ* fermentation production process (Table 3). Diffraction peaks for pure BC showed three distinct peaks at $2\theta = 14.8^\circ, 16.5^\circ$ and 22.8° corresponding to planes (1T0), (110) and (200), representing cellulose I crystal allomorph. Introduction of RGO during *in-situ* fermentation did not significantly change the crystal structure of BC in nanocomposites. However, the crystallinity indices (C.I) of BC/RGO nanocomposite films were found to be decreased from 81.2 % (for pure BC) to 80.4, 73.2, 70.8 and 65.4% when 1, 2, 3, and 5 wt. % RGO added. The appearance of a clear distinct peak at $2\theta = 26.5^\circ$ in all BC/RGO nanocomposites, confirmed the presence of uniformly dispersed RGO nanosheets entangled in between BC fibers (Figure S1). However, the presence of such interactions prevents the closed BC fibers packing, resulting in reduced crystallinity with increased functionality, which is required for improved interfacial interaction as confirmed from XRD studies.

The surface functionalities and structural changes with RGO addition during *in-situ* fermentation were analyzed by using Raman spectroscopy as seen in Figure 3 (a). A characteristic RGO Raman spectra showed two typical peaks at $1,349\text{ cm}^{-1}$ and $1,584\text{ cm}^{-1}$, representing D and G bands. G band is accredited to first order E_{2g} vibrations of C sp^2 atoms originating due to defects from oxygen deficiencies, grain boundary impurities or presence of amorphous carbon vacancies (Wu et al. 2018). The D band represents A_{1g} symmetry breathing mode of k-point phonon, corresponding to disordered RGO structure. Peak intensity ratios (I_D/I_G) measure the defect as well as crystallite domain size, providing details on structural changes of RGO sheets during *in-situ* biosynthesis. I_D/I_G ratio for BC/RGO nanocomposites show significant variation, decreasing from 0.98 (pristine RGO) to 0.81 and 0.72 for RGO content of 2 and 5 wt. %, respectively. It should be noted that the intensity of G-band almost remained constant, with sharp decrease in D band intensity. This decrease in I_D/I_G is possibly due to the reduction of partially oxidized RGO sites during *in-situ* biosynthesis of BC fibers, intercalating between graphene inter-layers. Consequently, the reduction in I_D/I_G suggests deoxygenation or graphitization of RGO which can occur during removal of oxygen species (from the hydroxyl or epoxy groups) present in RGO. Such reduction phenomenon can occur due to the presence of complex constituents present in growth media such as yeast extract or presence of BCs with high hydroxyl functionalities (acts as reducing agents) during *in-situ* biosynthesis, as observed by (Nandgaonkar et al., 2014). Earlier reported studies have shown that BCs acts as reducing agent for *in-situ* biosynthesis of silver (Pourreza et al., 2015), gold (Zhang et al., 2010), cadmium sulphate (Li et al., 2009) nanoparticles etc. Similarly, we expect that RGO undergoes gradual reduction during *in-situ* fermentation in presence of both media components as well as BCs, are the main possible reasons for the improved interaction and dispersion of RGO nanoflakes in between BC nanofibers. Interestingly, BC/RGO Raman spectra showed an additional peak at $\sim 2,701\text{ cm}^{-1}$, which represents the 2D band and provides additional information regarding stacked graphene layers. The intensity of 2D (I_{2D}) and G (I_G) bands, ratio of $\frac{I_D}{I_G} \sim 2 - 3$ suggests the presence of graphene monolayer, and $\frac{I_D}{I_G} \sim 0.8, 0.3$ or 0.07 signifies bi-, triple- and multi-layered graphene nanosheets (Krishnamoorthy et al. 2013). In current study, the ratio varied from $\frac{I_D}{I_G} \sim 0.72 - 0.62$ for 2 and 5 wt. % of RGO, suggesting that the layer-by-layer structure of RGO comprising bi- or triple layers of nanosheets, is still prevalent in *in-situ* prepared nanocomposite. It could be concluded, that RGO reduction

during *in-situ* BC/RGO biosynthesis in presence of hydrogen bond, helps in effective intercalation of BC fibers into RGO at mono-layer scales that resulted in improved physicochemical properties, as discussed subsequently.

Pore structure and surface area analysis of freeze-dried BC/RGO nanocomposites were carried out via nitrogen physio-sorption measurements to study the effective dispersion of GO in BC network. It was observed that introduction of RGO increases BET surface area from 11.5 m²/g (for pristine BC) to 42.1 and 47.5 m²/g at 1 and 2 wt. % of RGO (Table 3). Moreover, the average pore size distribution shows a similar trend which reduced at higher RGO loading with maximized being at 2 wt. %. This confirms that RGO intercalated within 3D BC fibers network is well dispersed and still maintains its two or three-layered nano-dimensions, as also confirmed from Raman spectroscopy studies. However, higher RGO loadings (> 3wt. %) decreased the surface area to 35.4 m²/g, suggesting possible agglomeration of nanosheets which reduces the porous architecture of BCs. The nitrogen adsorption-desorption curve shows type IV isotherm characteristics (Figure 3(b)) with hysteresis loop extending up to P/P₀~1. Pore size distribution curves show the presence of major fractions of mesopore and micropores originating from enhanced RGO dispersion in between BC fibers which act as scaffolding support, thereby increasing overall porosity of nanocomposites.

RGO with negative charge density and hydrophobic surface has a profound effect on bacterial kinetic behavior and on macromolecular properties of BC fibers produced during *in-situ* biosynthesis. The investigation of weight average degree of polymerization (DP_w) and molecular weight (M_w) for pristine BC was found to be ~ 2,450 and ~441 kDa. Presence of RGO during *in-situ* fermentation shows slight increase in M_w and DP_w at higher RGO concentrations. M_w and DP_w were increased to ~468 kDa and ~2,600 in presence of 2 wt. % RGO. This suggests that the presence of RGO in culture media has a synergistic effect in facilitating the cellulose biosynthetic pathway with improved bacterial metabolic activities. Obviously, at higher RGO loadings (>3wt. %), both M_w and DP_w were unaltered in range of 455-462 kDa and 2,500. The production of BC/RGO composites using glucose as carbon source didn't show any significant difference in M_w ~420-510 kDa, with respect to sugarcane hydrolysates as carbon source (Table 2). Conclusively, *in-situ* BC/RGO has a favorable effect on M_w properties which will be useful to study morphological and structural properties of BC/RGO nanocomposites, as discussed in the next section.

Table 3: Evaluation of the physicochemical properties of *in situ* produced BC/RGO films.

Properties	BC	BC-1RGO	BC-2RGO	BC-3RGO	BC-5RGO
Crystal Properties					
Crystallinity Index (C.I)	81.2	80.4	73.2	70.8	65.4
Surface Area Properties					
BET surface area (m ² /g)	11.5	42.1	47.5	36.6	35.4
Avg. pore size (nm)	10.3	12.6	12.0	9.7	8.5
Total pore volume (cc/g)	0.12	0.18	0.32	0.23	0.21

Physical Properties					
Molecular Weight (M_w)	441,025	440,050	468,020	462.500	455.234
Degree of Polymerization (DP_w)	2,450	2,445	2,600	2,570	2,530

Figure 3: (a) Raman spectroscopy and (b) Nitrogen adsorption-desorption isotherm of BC/RGO films produced through *in situ* fermentation.

3.5 Morphological characteristics of *in-situ* BC/RGO composite films

RGO distribution and morphology of fibers produced during *in-situ* fermentation were studied by using FESEM (Figure 4). Dried pristine BC films appear whitish that turn into black (highly flexible) with incorporation of RGO (Figure 1 (inset)). The pristine BC shows a highly dense cellulosic nanofibrils network (dimensions (width) 35 ± 8 nm), which forms uniform mat-like structure (Figure 4 (a)). It should be noted that BC fibrils generally have a high aspect ratio, entangled in form of mesh without any chain ends because of which BC nanofibers dimensions are reported in terms of width/diameter. The BC/RGO film micrographs with RGO 1 wt. % shows the presence of RGO nano-flakes uniformly dispersed in between BC fibrils. However, RGO sheets were found to be uniformly dispersed, with increased RGO loading up to 2 wt. %, which closely wrapped BC nanofibers to form a three-dimensional percolated network. The high-resolution FESEM micrographs (Figure 4(d)), shows the presence of dense BC nanofibrils network inter-woven with RGO nanosheets forming a continuous system with high uniformity and smoothness. The presence of such improved interfacial linkages between two discrete systems is

possibly due to intra-molecular hydrogen bondings and hydrophobic interactions resulting from *in-situ* RGO reduction. Morphological investigations of BC-2RGO film cross-section shows the presence of alternately stacked RGO layers intercalated with BC fibrils formed through a layer-by-layer approach at air-water interface (Figure 4(e)). High-resolution Figure 4(f) shows that the BC nanofibers form a web-like structure interlinking different layers, forming a three-dimensional network, similar to artificial nacre formed via chemical-based approaches. It was also observed that the dimensions of nanofibrils changes with RGO concentration, to form thinner BC fibers of diameter 31 ± 10 nm and 25 ± 9 nm at 1 and 2 wt.% RGO loadings, respectively. However, morphological characteristics of BC/RGO remain constant. Thinner fibrils have higher aspect ratio which tends to provide more entanglements sites with RGO which is expected to improve the reinforcement capability of nanocomposites. With an increase in RGO concentration (>3 wt. %), the RGO sheets get closely packed to form lumps within thin BC nanofibrils network and fill the interfacial sites. Further, at higher loadings (5 wt.%), it was observed that RGO nanosheets get agglomerated with the formation of voids and discrete zones of separate bundle of BC fibers and RGO flakes. Due to higher agglomerations, the dimensions of BC fibrils increase to 42 ± 6 nm and 38 ± 10 nm for 3 and 5 wt. % RGO content respectively. The BC/RGO fibers produced using glucose as sole carbon source were found to be of similar morphology but with relatively thinner cross-sections (Figure S2). From the morphological investigations, it could be concluded that *in-situ* BC/RGO nanocomposites biosynthesis shows a transition from segregated to percolated to aggregated network-like structures which are found to significantly depend on the concentrations of RGO used.

Figure 4: FESEM micrographs of (a) pure BC films, top view of (b)BC-1RGO, (c)BC-2RGO, (d) high resolution micrographs of BC-2RGO for selected region, (e)cross-sectional view of BC-2RGO films, (f) high resolution micrographs for selected region in BC-2RGO, (g) BC-3RGO and (h)BC-5RGO nanocomposites produced through *in-situ* fermentation approach utilizing sugarcane straw as feedstock.

3.6 Mechanical properties of the in-situ BC/RGO nanocomposite biosynthesis

The RGO dispersion into BC network resulted in three different states of distribution (Figure 5 (a)) that considerably affect the mechanical behavior of nanocomposite films. Pristine BC films showed an ultimate tensile strength (UTS) of 61 MPa and elongation of 8 %, which is similar to earlier reported studies that depend on carbon source type used during fermentation (Mohammadkazemi et al. 2015). The UTS of BC/RGO nanocomposites was enhanced by 41% (at 3wt. % of RGO), with a decrease in elongation to 5%. Young's modulus (YM) of BC/RGO nanocomposites were calculated to study the effect of RGO on the flexibility of composite films. The YM of BC-2RGO films was improved by 3 orders of magnitude as compared to pristine BC and remained almost unchanged (improvement by 2 orders of magnitude) even at higher RGO concentration. The improvement in YM of BC/RGO produced using sugarcane hydrolysates is comparatively higher than that produced using glucose as sole carbon source. This is possibly due to the presence of trace quantities of lignin residues which have improved interaction with RGO resulting in stronger reinforcing effect in developed nanocomposites. Also, the BC and BC/RGO films produced from the pure glucose as sole carbon source shows lower YM values (as shown in Figure in the supplement). The presence of the lignin in the trace quantities in the biomass hydrolysates due to their improved interfacial interaction with RGO and BC fibers acts as a strong reinforcing agent. Interestingly, only one kind of mechanical property has been frequently improved in the literature (Zhu et al. 2015). However, simultaneous improvement in both tensile stress and toughness remains to be a great challenge. The integrated improvement is possibly due to strong interfacial interaction between BC and RGO interlinkages to absorb energy at higher stress. The toughness was improved by 65% in presence of 1wt. % RGO, when compared with pristine BC, which was decreased thereafter with increased RGO content, however still higher by 28, 16 and 6% at 2, 3 and 5 wt. % RGO loadings. The improvement in both tensile strength and toughness, could be addressed due to high surface energies of BC fibers and RGO nanosheets. It results in strong integration of two systems with their improved dispersion at nanoscale dimensions, as also concluded from morphological investigations. The presence of such interfacial forces results in the effective transfer of RGO modulus to BC fibers with inherent flexibility that results in higher toughness. Interestingly, enhancement in YM and toughness was observed in 1-2 wt. % RGO loading, suggesting that improved filler-matrix interaction along with formation of jammed percolated-network was dominant in nanocomposites. The, percolation theory based on the series-parallel model proposed by (N Ouali, 1991) obtained from parallel-series model was studied (Eq. (3)) to determine the rigidity of BC/RGO nanocomposites.

$$E_c = \frac{(1 - 2\psi + \phi\psi)E_mE_f + (1 - \phi)\psi E_f^2}{(1 - \phi)E_f + (\psi - \phi)E_m} \quad (3)$$

$$\text{where, } \psi = 0 \quad \phi \leq \phi_c$$

$$\psi = \left(\frac{\phi - \phi_c}{1 - \phi_c} \right)^b \quad \phi > \phi_c$$

where the ϕ is the filler volume fraction, E_m , E_f and E_c are shear moduli of the matrix, fiber or filler and the prepared composite and ψ is volume fraction of percolating filler network. In order to convert the weight percent to volume fraction, the density of RGO was considered to be $\rho \sim 2.23 \text{ g/cm}^3$ (Feng et al. 2017), and the calculated RGO volume fraction are ~ 0.45 , 0.90 , 1.34 and 2.24 vol. % for 1, 2, 3 and 5 wt. % of RGO. In case of BC with the presence of highly

entangled fibrillar network, the effective stress transfer to composite is due to their backbone structure, therefore, the value of b is assumed to be ~ 0.8 , as per earlier reports (Gennes, 1976) (Nawaz et al. 2012). From the mechanical percolation model, it could be seen that the modulus didn't show any appreciable increase at 2 wt. % of RGO (Fig. 5). However, the mechanical behavior was significantly improved at $\text{RGO} > 2 \text{ wt. \%}$, following a power law. This implies that $\text{RGO} < 2 \text{ wt. \%}$ initiates to form a percolated network-like structure due to molecular forces present in between BC and RGO platelets, which strengthens at higher loadings (Figure 6(a)). It can be observed that above the critical threshold concentration of RGO ($> 2 \text{ wt. \%}$) the experimentally determined YM follows the trend as predicted through percolation model (Figure 5(d)). However, the experimentally determined mechanical properties overshoots the values predicted from percolation model. This confirms that the percolative network formation is due to strong interactive forces such as intermolecular hydrogen bonding and *in-situ* reduction of RGO sheets as discussed in earlier section. However, these models don't take into consideration the effect arising from the molecular level interactions such as inter or intra-molecular hydrogen bondings which are generally present in cellulose fiber system (Dhar et al. 2015; Hossain et al., 2012). In the present system of BC and RGO, two main factors are responsible for high YM, firstly, (i) BC have highly entangled fibrillar network and secondly (ii) strong intermolecular hydrogen bonding arising from *in-situ* reduction of RGO sheets and BC produced during fermentation, induces stronger reinforcements than predicted by mechanical models. The proposed *in-situ* fermentation approach in this study results in the development of BC/RGO nanocomposites with enhanced strength as well as toughness, which have great promising applications.

Figure 5: Mechanical property of BC/RGO films at various RGO loadings (a) Ultimate Tensile Strength (UTS), (b) Young's Modulus (YM), (c) Toughness and (d) Experimentally determined tensile modulus fitted with classical percolation model and determination of critical threshold percolation concentration (marked with arrow).

3.7 Electrical conductivity properties of in-situ produced BC/RGO nanocomposite films

As observed from morphological studies, films fabricated with percolated network-like structures and layered assembly of RGO/BC is expected to improve the electrical conductivity, which is reported in Table 4. Pristine BC films due to the absence of any conductive moieties showed extremely poor electrical conductivity of $\sim 8.5 \times 10^{-5}$ S/cm, as expected. Incorporation of RGO at 1-2 wt. % increased the electrical conductivity by ~ 6 to 7 orders of magnitude (nearly ~ 100 S/cm). Further increase in RGO content (up to 5 wt. %) continued to increase the electrical conductivity with steady-state afterward (Table 4). During *in-situ* BC/RGO biosynthesis (at 3 wt. % RGO); RGO sheets were effectively wrapped onto BC surface due to the presence of intra-molecular hydrogen bondings. Additionally, a gradual reduction of RGO sheets was also observed from spectroscopic studies, which might have possibly resulted in high conductivity values. These high electrical conductivity values of ~ 140 -150 S/cm for BC/RGO films are adequate for their practical applications in electronics as free-standing films or paper-based electronics. Chemical routes for fabrication of conductive BC nanocomposites have been extensively studied utilizing nanofillers such as polypyrrole, carbon nanotubes or polyaniline for potential applications in electrodes or as supercapacitors. It was observed that the current-voltage characteristics of BC/RGO films can be measured using house-hold multimeter (Figure 1 (inset)), suggesting their promising application in replacement of fossil-based conductive polymers. The reported conductivity of BC/RGO films produced via inset biosynthesis approach using agricultural residues as a feedstock is certainly comparable with BC nanocomposites produced via chemical modification routes.

To further confirm the analogy on the presence of percolated network-like structures in BC/RGO films, the electrical conductivity values were modeled using power law or scaling law equation (Nassira et al., 2016) (Eq. 4).

$$\sigma_c = \sigma_f(\rho - \rho_c)^r \quad (4)$$

where the σ_c represents the conductivity of composites, σ_f conductivity for filler, ρ concentration of RGO and ρ_c concentration of RGO at percolation threshold. The universal critical exponent (r), is a parameter which determines the type of network prevailing in a composites depending on its values: (i) $r \sim 1$ suggests 2D percolated conductive system of micro/nanofillers, (ii) $r \sim 1.3$ represents presence of continuum networks and (iii) $r \sim 2$ confirms the presence of three dimensional conductive percolated systems. Using the classical theory, the value of universal critical exponent (r) was calculated to be ~ 2.25 and critical percolation threshold concentration of RGO to be (ρ_c) ~ 1.58 wt. %. From the values of universal critical exponent (r), it could be confirmed that RGO nanosheets distributes itself into 3D hierarchical organization forming interconnects (Figure 4 (e)), which results in enhanced conductivity. As per earlier reported studies (Debbarma et al. 2016), the presence of percolated architecture in conductive films is of significant importance for the development of components in electronic devices. The formation of highly conductive BC/RGO nanocomposites by using agricultural feedstock opens up new avenues in bio nanocomposite area. It should be noted that fermentation is a dynamic process varying with time, due to which the characteristics of percolated-network formed can be tuned depending upon the targeted applications. The present study reports a unique approach of fabricating highly conductive BC/RGO films through an *in situ* fermentation approach utilizing sugarcane straw residues, converting the waste by-products to value-added products for technological applications. We envision that renewable feedstocks such as sugarcane residues contains a large fraction of fermentable sugars with adequate quantities of xylose, acetic acid or

lignin derivatives is expected to boost the BC production, which can be smartly selected for bioprocessing of renewable biomass wastes into value added products.

Figure 6: (a) Schematic diagram of percolated network-like structure formation with increasing RGO concentration, (b) measured electrical conductivity values fitted with the percolation model and (c) linear fitted logarithmic plot of conductivity versus $(\rho - \rho_c)$ to determine universal critical exponent $r \sim 2.25$ and percolation threshold concentration of ~ 1.58 wt. % of RGO.

Table 4: Electrical properties of the BC/RGO films produced during *in-situ* fermentation.

Samples	Sheet Resistance (Ω^{-1})	Thickness (μm)	Specific Resistivity ($\Omega.\text{cm}$)	Conductivity (S cm^{-1})
BC	1.2×10^9	9.2	1.1×10^4	$8.5 \pm 1.1 \times 10^{-5}$
BC 1RGO	7.5×10^2	8.3	0.625	1.6 ± 0.2
BC 2RGO	9.52	10.5	0.01	98.2 ± 15.8
BC 3RGO	7.30	9.6	7×10^{-3}	142.1 ± 20.5
BC 5RGO	7.64	8.5	6.5×10^{-3}	152.0 ± 18.1

4. Conclusions

The present study utilizes sugarcane straw as agricultural waste to produce highly conductive BC/RGO nanocomposite films via *in-situ* fermentation strategy. The sugarcane straw was pretreated with liquid hot water treatment followed by enzymatic hydrolysis to release fermentable

sugars. These fermentable sugars were explored to produce *in-situ* modified BC/RGO nanocomposites at different RGO concentrations. The fermentation kinetic studies showed that RGO concentrations governs the process parameters and overall yield, by reaching maximum values at 2wt. % RGO loadings. The BC/RGO films were found to be highly crystalline, with an improved degree of polymerization and high surface area which was found to be governed by RGO content. The BC nanofibers with intercalated RGO nanosheets showed the presence of highly integrated and entangled networks resulting in formation of hierarchical layer-by-layer architecture. Interestingly, the mechanical properties and electrical conductivity of BC/RGO showed the presence of percolated network threshold at 1.5 wt.%, as evident from classical percolation theory models. Additionally, *in-situ* BC/RGO films also showed positive improvements in both tensile moduli as well as toughness, which is otherwise difficult to achieve through chemical-based nanocomposite formation. Finally, *in-situ* BC/RGO films showed high electrical conductivity of ~142 S/cm (at 3wt. % RGO loadings), which is suitable for practical applications in flexible electronic devices, as demonstrated in this study. The present study addresses the challenges related to BC nanocomposite bioprocessing by utilizing cheap and abundantly available sugarcane straw hydrolysates.

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