Fabrication of hybrid thin film based on bacterial cellulose nanocrystals and metal nanoparticles with hydrogen sulfide gas sensor ability

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ARTICLE INFO

Keywords:
Bacterial cellulose nanocrystals
Acid hydrolysis
Silver nanoparticles
Molybdenum trioxide nanoparticles
Hybrid material film
Hydrogen sulfide gas sensor

ABSTRACT

The nanocrystalline structures of bacterial cellulose (BC) are described as “environmentally friendly green nanomaterials”. Bacterial cellulose (BC) was produced from Gluconacetobacter xylinus in pellicle form with a large bundle of fibers were acid hydrolyzed to obtain bacterial cellulose nanocrystals (BCNCs). The H\textsubscript{2}SO\textsubscript{4} acid-hydrolyzed BCNCs were evaluated for their smallest crystalline size and hydrodynamic size, highly negative zeta-potential value, and the highest specific surface area to interact with metallic nanoparticles. Hybrid thin film of BCNCs based surface-loaded silver nanoparticles (AgNPs) and alginate-molybdenum trioxide nanoparticles (MoO\textsubscript{3}NPs) was developed for hydrogen sulfide (H\textsubscript{2}S) gas sensor. Sensor characteristics were investigated as well as its response with H\textsubscript{2}S gas. The film was successfully detected H\textsubscript{2}S gas. The color of the film changed by the shift of oxidation number of MoO\textsubscript{3}NPs. Once activated by AgNPs, MoO\textsubscript{3}NPs was readily reduced to a colored sub-oxide by atomic hydrogen that produced and received from reaction of H\textsubscript{2}S gas.

1. Introduction

Almost ten years of advances in natural-based or bio-based polymer research have proved the potential and importance of biopolymers for a wide variety of applications, specifically for biopolymers formed by microorganisms or microbes, including bacterial cellulose nanocrystals (BCNCs) (Jozala et al., 2016; Lin, Huang, & Dufresne, 2012; Ummartyotin & Manuspiya, 2015). Apart from plant-derived cellulose, certain microbial-derived cellulose or bacterial cellulose produced by Gluconacetobacter xylinus, tunicate, and algae are also known to produce cellulose in considerable quantities in a relatively pure form even though the chemical structure is similar (George, Ramana, Bawa, & Siddaramaiah, 2011; Shah, Islam, Khattak, & Park, 2013). Bacterial cellulose (BC) has unique properties because of its high purity (no hemicellulose or lignin), higher surface area as compared to native cellulose, high crystallinity, high water swelling, moldability, good shape retention, excellent mechanical and thermal properties (Ashjaran, Yazdanshenas, Rashidi, Khajavi, & Rezaee, 2013; Corrêa, de Morais Teixeira, Pessan, & Mattoso, 2010; Fu, Zhang, & Yang, 2013; George et al., 2011; Jozala et al., 2016; Lin & Dufresne, 2014; Shah et al., 2013; Singh, Narain, & Manuspiya, 2018; Tang, Sisler, Grishkewich, & Tam, 2017; Vasconcelos et al., 2017).

In the area of nanomaterials technology, the top-down approaches (e.g.: homogenization, hydrolysis, combined chemical-mechanical processes) can be applied to downsize the plant-derived cellulose or microbial-derived fibers in small particles as cellulose nanocrystals (CNCs) suspensions, expanding versatility and new nanomaterial resources to this cellulosic material (Lin et al., 2012; Vasconcelos et al., 2017). These CNCs have unique nano-porous three-dimensional networks at nanometric scales (Fu et al., 2013), and they dominate various attractive characteristics, such as large surface area, easy surface modification, and high aspect ratios which can facilitate the penetration of inorganic nanoparticles or metallic ions into the CNCs structure.

Acid hydrolysis, especially in the strong inorganic acids, such as H\textsubscript{2}SO\textsubscript{4} and HCl are the most commonly used method for producing CNCs (Dufresne, 2012) by penetrating of hydrogen ions (H\textsuperscript{+}) into amorphous cellulose molecules promoting cleavage of covalent or glycosidic linkage/bonds, therefore releasing single crystallites (Vasconcelos et al., 2017). H\textsubscript{2}SO\textsubscript{4} produces a highly stable colloidal suspension because of the high negative surface charge contributed by sulfonation of the CNCs surface. However, the presence of sulfate groups (–OSO\textsubscript{3}–) reduces the thermal stability of the nanocrystals (Martínez-Sanz, Lopez-Rubio, & Lagaron, 2011). In contrast, when HCl is used, a low-density surface charges is produced on the CNCs with limited nanocrystal dispersibility, which tends to contribute sediment in aqueous suspensions. Some studies describe the companion use of...
H2SO4 and HCl to generate stable and thermally resistant CNCs suspensions (Corrêa et al., 2010). CNCs with different physical and mechanical properties can be yielded by the acid hydrolysis reaction conditions, which depend on the concentration and type of acid, reaction time, and temperature. Many types of BCNCs have been developed for numerous applications, including textile industry, nonwoven cloth, paper, food, waste treatment, broadcasting, mining, refineries (Ashjaran et al., 2013; Lin & Dufresne, 2014; Wu & Liu, 2012), drug delivery systems, vascular grafts, and scaffolds for tissue engineering in vitro and in vivo (Jozala et al., 2016).

Hydrogen sulfide (H2S) gas is a colorless, flammable, extremely hazardous gas with the characteristic of a “rotten egg” smell (Chen, Wang, Hartman, & Zhou, 2008; Schirmer, Heir, & Langsrud, 2009). It often results from the microbial breakdown of organic materials in the absence of oxygen. Since, it is a chemical suffocant, which could cause to lose consciousness even at a very low concentration, paralyze the lungs and affects human’s nervous system (Choudhary, Singh, Mishra, & Dwivedi, 2013; Gahlaut, Yadav, Sharan, & Singh, 2017; Malone Rubright, Pearce, & Peterson, 2017; Pandey, Kim, & Tang, 2012), and at a very high concentration (> 700 ppm) it could cause death by reducing the oxygen-carrying capacity of the blood, by interrupting the oxidative processes of tissue cells, and by paralyzing the nervous system leading to respiratory failure (Chen, Morris, & Whitmore, 2013; Gahlaut et al., 2017). According to the potential hazardous nature of H2S gas, it is important to detect for both human health and industrial control purposes, especially in the terms of raw meat products of food packaging industry (Sarfraz et al., 2014). Moreover, H2S gas is one of the most often encountered food spoilage gases in meat packaging systems, which is generated by the reason of microbial spoilage and lipid oxidation (Murphy, O’Grady, & Kerry, 2013) can change the headspace gas composition in the package headspace (Coombs, Holman, Friend, & Hopkins, 2017; Mohebi & Marquez, 2015). Accordingly, many smart packaging technologies based on sensor label have been explored during recent decades. The sensor label could be in the form of smart labels, immobilized, or printed on/inside the packaging films, which would enable to detect the level of gaseous or volatile organic compound (VOC) content increases during the spoilage either by bacteria or enzymic degradation (Brockgreitens & Abbas, 2016; Coombs et al., 2017; McMillin, 2017; Remenant, Jaffrès, Dousset, Pilet, & Zagorec, 2015; Sofos, 1994) and caution about the quality and safety of the meat products by changing in their color or visible spot.

Recently, the use of hybrid material, which are consisted of two constituents at nanometric scale. Commonly, in the organic–inorganic matrices, these materials revealed a significant improvement in mechanical properties of nanocomposites consisting of nanoparticles dispersed within the matrix phase for expanding the application range of these materials (Gonçalves et al., 2013). For the use of inorganic materials in association with almost any hybrid materials were reported and developed for volatile substances and gases sensor. Some recently reported the integration ofcellulosic materials with metal oxides with the aim of preparing a hybrid or composite allowing high accessibility to more or less aggregated nanoparticles of incorporation with the oxide (Shimizu, Imai, Hirashima, & Tsukuma, 1999). Moreover, bacterial cellulose, has been revealed to be a potential to offer isotropic 3D nano-scaffold for perfect being host these inorganic compounds at the nano-scale, resulting in improvement of the inorganic phase activity, ascribed to the use of BC with high specific surface area (Foresti, Vázquez, & Boury, 2017). For example, the simplest way to achieve the integrated BC and metal-oxide nanoparticles is to mix them under vigorous stirring (Zhang et al., 2016). Besides, a number of semiconductors, such as MoO3 (Kabcum et al., 2016; Li et al., 2017; MalekAlaie et al., 2015), SnO2 (Kabcum et al., 2016; Sarala Devi, Manorama, & Rao, 1995), α-Fe2O3 (Kersen & Holappa, 2006), ZnO (Wang, Chu, & Wu, 2006), In2O3 (Xu, Wang, & Shen, 2006), WO3 (Lin, Hsu, Yang, Lee, & Yang, 1994; Solis, Saukko, Kish, Granqvist, & Lantto, 2001), and CuO (Chen et al., 2008) have been investigated to detect and to monitor trace concentrations of H2S. Among all reported sensor-based semiconducting metal-oxide, MoO3 based gas sensors are most widely investigated because of its small size, high sensitivity, fast response, simple construction, and low cost, which has plenty of Lewis-acid sites, exhibits a high reactivity to H2S, probably because of its high chemical affinity to H2S (Kabcum et al., 2016; Li et al., 2017; MalekAlaie et al., 2015). However, a very fast and sensitive initial response of silver nanoparticles (AgNPs) and H2S gas was very interested and reported to be a first-order reaction in AgNPs, and the initial reaction rate was proportional to the gas concentration down to the 1 ppmv volume (ppmv) level (Chen et al., 2013). Hence, the sensitivity of the H2S detection using two different classes gas sensor, AgNPs fast reacted directly with H2S to generate atomic hydrogen and MoO3NPs will be reduced to a colored sub-oxide by atomic hydrogen, which will become especially interesting in the improvement of hybrid material for H2S gas sensor.

The aim of this work was to present the design of bacterial cellulose nanocrystals and metal nanoparticles based polysaccharide sodium alginate matrix as a hybrid thin film for H2S gas sensor. The effect of acid hydrolysis on bacterial cellulose nanocrystals preparation was investigated. Preliminary experiments with H2S gas exposure at room temperature with time-varying have been performed. The different concentrations of H2S gas, especially at higher concentration, of course, reduce more Mo oxidation state, but this is out of the scope of this manuscript.

2. Materials and methods

2.1. Materials

K. xylinus strain (TISTR No. 975) was purchased from the Microbiological Resource Center, Thailand Institute of Scientific and Technological Research (TISTR) (http://www.tistr.or.th/mircen/index.html). D-glucose anhydrous, yeast extract, peptone, and calcium carbonate (CaCO3), sulfuric acid (H2SO4), hydrochloric acid (HCl), ACs reagent, 37 %), Molybdenum (VI) oxide nanopowder (MoO3NPs), sodium borohydride (NaBH4), sodium alginate, sodium hydroxide (NaOH), and zinc chloride (ZnCl2) were obtained from Sigma-Aldrich. Silver nitrate (AgNO3) was obtained from Merck Chemicals. Sodium sulfide nonahydrate (Na2S·9H2O, > 95 %) was purchased from Ajax Fine Chem, Co, LTD, Thailand. Ultrapure water from a Milli-Q water was used for all of the experiments. All chemicals were used as received.

2.2. Production of bacterial cellulose (BC)

The BC hydrogel-like pellicle, biosynthesized by K. xylinus strains, was prepared by the partially modified method of (Janpetch, Saito, & Rujiravanit, 2016; Maneerung, Tokura, & Rujiravanit, 2008; Singhsha et al., 2018a). K. xylinus strains was cultured on agar containing 100 g of D-glucose anhydrous, 10 g of yeast extract, 5 g of peptone, 20 g of CaCO3, and 25 g/L of agar at a temperature of 30 °C for 3 days. A sterilized 100 mL glucose yeast extract nutrient broth (GYNB) solution consisted of D-glucose anhydrous 5.0 % (w/v) and yeast extract 1.0 % (w/v) in Milli-Q ultrapure water was prepared to suspend two ampoules of the freeze-dried cells of K. xylinus (TISTR No. 975). The cell suspension was incubated at a temperature of 30 °C for 24 h. The 50 mL of bacteria inoculum was introduced into 2 L of GYNB and incubated at 30 °C for 7 days under static conditions. After incubation, the BC pellicle was produced on the surface of culture GYNB and then rinsed with distilled water to remove any residual GYNB. The acquired BC pellicle was purified by boiling in 2 % (w/v) NaOH solution at a temperature of 80 °C for 1 h and then rinsed repeatedly several times with deionized (DI) water to gain the purified neutral BC pellicle.
2.3. Preparation of bacterial cellulose nanocrystals (BCNCs)

The purified BC pellicles were mechanically disintegrated to a BC paste using a laboratory blender at 5000–6000 rpm for about 15 min under ambient temperature. The BC paste was filtered through filter paper (Whatman No. 1) to remove excess water prior to acid hydrolysis. BCNCs were prepared through acid hydrolysis. The BC paste was hydrolyzed with acid solution in a ratio of 1:20 g/mL with continuous stirring in different conditions as follows: the 65 % H2SO4 solution with time-varying for 1, 2, and 3 h at 45 °C for H2SO4 hydrolysis by the partially modified method of (George et al., 2011) and the 4 N HCl solution with time-varying for 4, 5, and 6 h at reflux temperature for HCl hydrolysis (designated as BCNCs-1 to BCNCs-6) (see Table S1).

The hydrolyzed BCNCs suspension from each acid hydrolysis reactions were then added an excess of DI water (5-fold) and cooled with an ice bath to terminate acid hydrolysis reaction. The excess acidic solution was removed by centrifugation at 10,000 rpm at 4 °C for 10 min for precipitating the BCNCs and then poured out the excess acidic solution. The precipitated BCNCs was collected and dialyzed (MWCO > 12,000) against DI water to gain the neutral BCNCs. After that, the neutral BCNCs was centrifuged by centrifugation at 10,000 rpm at 4 °C for 10 min. Finally, the BCNCs was removed an excess water by freeze-drying at −55 °C for 24 h to collect the dried-BCNCs.

2.4. Preparation of BCNCs based surface-loaded AgNPs (BCNCs–AgNPs)

Freeze-dried BCNCs surface were pre-loaded with silver ion (Ag+ ) by immersing in 1 mM of the aqueous AgNO3 under ultrasonic treatment by using a high intensity ultrasonic bath (GT SONIC-D3) at frequency of 40 kHz for 3 h. After an ultrasonic treatment in AgNO3, BCNCs–Ag+ were then rinsed with ethanol for ca. 30 s to eliminate the Ag+ that were unadhered and loosely adhered to the BCNCs surface. After that, the Ag+ saturated–BCNCs were ion-reduced in 100 mM of the aqueous NaBH4 (NaBH4:AgNO3 molar ratio of 100:1) for 20 min and rinsed several times (5 min each) with ultra-pure water to eliminate all traces of chemicals, by the partially modified method of (Maneering et al., 2008). Finally, the obtained samples were frozen at −40 °C and freeze-dried at −55 °C for 24 h to get BCNCs–AgNPs samples.

2.5. Preparation of alginate–MoO3NPs solution

A 4 wt% homogeneous sodium alginate solution was prepared by dissolving sodium alginate powder in ultra-pure water at 60 °C for 4 h under magnetic stirrer at a stirring speed of 1000 rpm. In parallel, MoO3NPs solution was prepared by dissolving required amount of 100 mM in ultra-pure water at room temperature for 2 h under ultrasonic treatment by using a high intensity ultrasonic bath (GT SONIC-D3) at frequency of 40 kHz. Half the volume of the 4 wt% homogeneous sodium alginate and 100 mM MoO3NPs solution were mixed at room temperature for 2 h under magnetic stirrer at a stirring speed of 800 rpm to reach a final concentration of 2 wt% alginate–50 mM MoO3NPs solution.

2.6. Preparation of hybrid BCNCs–AgNPs and alginate–MoO3NPs film

The hybrid BCNCs based surface-loaded AgNPs and alginate–MoO3NPs film was prepared by immersing 0.1 dry wt% of the freeze-dried BCNCs–AgNPs in the 2 wt% alginate–50 mM MoO3NPs solution at room temperature for 1 h under magnetic stirrer at a stirring speed of 800 rpm. After that, the homogeneous BCNCs–AgNPs/alginate–MoO3NPs solution was appeared with a very light-grey color, was poured in a round-shaped poly(tetrafluoroethylene) (PTFE) or teflon mold (10 mm height; 4 mm depth; 20 mm diameter), then allowed to air-dry at ambient temperature for 48 h to obtain the BCNCs–AgNPs/alginate–MoO3NPs film samples (20 mm diameter; thickness 90 μm) and stored under dry conditions.

2.7. Preparation of H2S gas samples and gas exposure experiment

The ZnS powder was prepared by a simple and efficient one-pot synthesis of sodium sulfide nonahydrate (Na2S·9H2O) and zinc chloride (ZnCl2), by the partially modified method of (Infahsaeng & Ummartyonit, 2017), as follow: 1 mol of ZnCl2·7H2O was prepared in 500 mL of DI water under magnetic stirrer at a stirring speed of 600 rpm for 30 min at temperature of 60 °C. In parallel, 1 mol of Na2S·9H2O was prepared in 500 mL of DI water under magnetic stirrer at a stirring speed of 600 rpm for 30 min at 60 °C, and then added to 1 mol of ZnCl2 solution under magnetic stirrer at a stirring speed of 600 rpm for 1 h at 60 °C. The stoichiometric of chemical reaction is

\[ \text{Na}_2\text{S}·9\text{H}_2\text{O (aq)} + \text{ZnCl}_2·7\text{H}_2\text{O (aq)} \rightarrow \text{ZnS} (s) + \text{Na}^+ + \text{Cl}^- \]

After that, the aqueous suspension of ZnS was rinsed for 10 min with methanol to eliminate all traces of chemicals, centrifuged at 10,000 rpm for 20 min at 4 °C to obtain the sediment ZnS, and then kept in oven at 100 °C for 24 h to obtain the ZnS powder. H2S gas was prepared at ambient temperature (25 °C) by the reaction of 50 mM ZnS powder with an aqueous solution of 100 mM HCl in (molar ratio 1:2) in a 500 mL tightly septum-capped glass bottle, which was flushed with nitrogen gas and stored at room temperature for 1 h. The excess HCl solution could prevent diffusion of generated H2S into the reaction solution (Carpenter, Rosolina, & Xue, 2017). The stoichiometric of chemical reaction is

\[ \text{ZnS} (s) + 2\text{HCl} (aq) \rightarrow \text{H}_2\text{S} (g) + \text{ZnCl}_2 (aq) \]

The prepared H2S gas was calculated the mass and conversed in a concentration of ppm by following Eqs. (1)–(3):

\[ PV = Nk_B T \]  
\[ n = N/N_A \]  
\[ m = nM_A \]

Where \( P \) is the standard ambient pressure (1.01325 x 10^5 Pa), \( V \) is the volume of gas container (5 x 10^-4 m^3), \( N \) is the gas molecules, \( k_B \) is the Boltzmann’s constant (1.38 x 10^-23 JK^-1), \( T \) is the standard ambient temperature (298.15 K for 25 °C), \( n \) is a number of H2S moles, \( N_A \) is Avogadro’s number (6.023 x 10^23), \( M_A \) is the molecular weight of H2S gas (34.08 g mol^-1), \( m \) is the mass of H2S gas. Standard temperature and pressure (STP) is defined as a condition of 100.00 kPa (1 bar) and 273.15 K (0°C), which is a standard of the International Union of Pure and Applied Chemistry (IUPAC). Thus, the concentration of H2S gas was calculated to be approximately 1400 ppm in the 500 mL tightly septum-capped glass bottle. The H2S gas was diluted 100 times by displaced 5 mL of the H2S gas using syringe plunger to each of the film sample bottles with the same bottle volume. Finally, each of the film sample bottles was contained the H2S gas in concentration of approximately 14 ppm. For the H2S gas exposure of BCNCs–AgNPs/alginate–MoO3NPs film samples. Prior to gas exposure, the film samples were prepared for different gas exposure time (5 min, 1 h, and 24 h) by placing in different of the 500 mL septum-capped glass bottle with an identifying mark. The film samples in the septum-capped glass bottle with an identifying mark were subsequently exposed to the H2S gas, as follow: insert a clean needle of the syringe into the septum at the top of the bottle cap, the gas pressure in the bottle was displaced into the syringe plunger, waited for the gas volume to reach 5 mL, and then transferred the gas to each of the film sample bottles.

2.8. Characterization

BC production was reported as the dry weight of cellulosic fibers within the volume of bacterial culture media in liter (g/L). The dry weight of BC pellicle was obtained by freezing at −40 °C for 24 h prior to freeze-drying technique at −55 °C for 24 h. The chemical structures of...
all samples were investigated by using an attenuated total reflectance fourier transform infrared (ATR-FTIR, Nicolet Nexus 670 FT-IR spectrometer). All spectra were scanned between 4000 and 650 cm\(^{-1}\) at a resolution of 32 cm\(^{-1}\). OPUS 5.5 software (Bruker Optics GmbH, Ettlingen, Germany) was used for IR spectral acquisition. The \(I_\alpha\) fraction of the bacterial cellulose samples were calculated by the following equations obtained from (Bi et al., 2014; Singhsa et al., 2018a; Singhsa, Narain, & Manuspiya, 2018), equation parameters proposed by Yamamoto, Horii, and Hirai (1996).:

\[
f_\alpha = 2.55 f_\text{IR} - 0.32
\]  

where \(f_\text{IR}\) can be calculated as \(A_\alpha/(A_\alpha + A_\beta)\) and \(A_\alpha\) and \(A_\beta\) are the integrated intensities from the absorbencies at corresponding wave number 750 and 710 cm\(^{-1}\), respectively.

XRD diagrams of dried pristine BC and dried BCNCs samples were recorded using a Rigaku model SmartLab 4800 diffractometer with a Cu K\(\alpha\) radiation wavelength (\(\lambda = 1.54\) Å), generated at a voltage of 40 kV and a filament emission of 30 mA. Samples were scanned from the \(2\theta = 5\) to \(80^\circ\) range at a scan speed of \(2^\circ/\text{min}\) and a scan step of 0.02°. The average crystallite size (\(L_{\text{hkl}}\)) was calculated based on XRD measurements. \(L_{\text{hkl}}\) was calculated from the following the Scherrer equation (Eq. (5)):

\[
L_{\text{hkl}} = \frac{K\lambda}{\beta_{\text{hkl}}\cos\theta_{\text{hkl}}}
\]  

Where \(\beta_{\text{hkl}}\) is the breadth of the peak of a specific phase (hkl) or is the full width at half maximum height (FWHM) for the diffraction angle 2\(\theta\) in radian, \(K\) is a constant that varies with the method of taking the full width at half maximum height (FWHM) for the diffraction angle 2\(\theta\) in radian, \(K\) is a constant that varies with the method of taking the breadth (K = 0.94), \(\lambda\) is the wavelength of incident X-rays (1.54 Å), \(\theta\) is the center angle of the peak (Bragg’s angle). The crystallinity (%) was calculated from the following Eq. (6):

\[
\text{Cr}(%)=\frac{(S_c/S_t)\times 100}{100}
\]  

where \(S_c\) is sum of net area and \(S_t\) is sum of total area.

The \(\xi\) potentials of all acid hydrolyzed-BC suspensions were characterized at 25°C by Malvern Zetasizer Nano ZSP (Malvern Instruments Ltd., GB).

The Z-discriminant function (Z-value) was calculated using the following Eq. (7) proposed by Wada, Okano, and Sugiyama (2001):

\[
Z = 1.693d_2 - 90.92d_2 - 549
\]  

where \(d_2\) is the d-spacing of the peaks at 20 near 15° (I\(\alpha\) (100) and I\(\beta\) (110)) and \(d_2\) is the d-spacing of the peaks at 20 near 17° (I\(\alpha\) (101) and I\(\beta\) (1–10)). The relative content of cellulose \(L_c/cellulose \ I_\beta\) ratio was calculated using the following Eq. (8) proposed by Wada, Kondo, and Okano (2003):

\[
f_{\text{BCK-c}} = -70.542d_2 + 37.583d_2 + 23.360
\]  

The hydrodynamic sizes of all acid hydrolyzed-BC suspensions were introduced into the viewing chamber using the integrated fluids capability by NanoSight NS500 (Malvern Instruments Ltd., GB) by the DLS technique. The reported value is an average of 3 measurements.

The total specific surface areas and micro-pore volume of selected samples were characterized by N\(_2\) adsorption at the temperature of liquid nitrogen applying the Brunauer-Emmett-Teller (BET) technique (Brunauer, Emmett, & Teller, 1938)) (BELSORP, mini-II nitrogen adsorptometer, Osaka, Japan). As a primary step the samples were subjected to a freeze-drying process, and then evacuated under ambient conditions for 24 h.

The surface morphology of the samples were characterized by field emission scanning electron microscopy (FE-SEM, Hitachi S-4800 model). The freeze-dried pristine BC, freeze-dried BCNCs, and freeze-dried BCNCs–AgNPs were sputter-coated with platinum in preparation for FE-SEM imaging. A Hitachi model S-4800 FE-SEM microscope was used operating at an accelerated voltage of 5 kV and a magnification of 20 \(\times\). SEM/EDX was used for the determination of elemental composition of the freeze-dried BCNCs–AgNPs and the hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film. Transmission electron microscopy (TEM) micrographs of the pristine BC, BCNCs, and BCNCs–AgNPs samples were taken in a JEOL 100CX-2 transmission electron microscope at an accelerating voltage of 100 kV. Samples were diluted and prepared by dropping the sample suspension on a carbon-coated grid and allowed to dry, followed by staining with a 2 wt % aqueous uranyl acetate solution. Chemical compositions, oxidation state number of MoO\(_3\)NPs on the hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film were characterized by x-ray photoelectron spectroscopy (XPS, Kratos Axis Ultra DLD) and with a monochromatic Al K\(\alpha\) as an X-ray source (anode HT = 15 kV) and energy dispersive x-ray analysis (EDX, JSM-7610 F, JEOL). The depth of penetration of XPS is 10 nm.

The binding energies were referenced to the hydrocarbon C 1s peak at 284.6 eV (Susi, Pichler, & Ayala, 2015). Data evaluation was performed using CasaXPS. For fitting of the Mo 5d states, we employed the following parameters (Borgschulte et al., 2017; Ji et al., 2013):

\[
\begin{align*}
\text{Mo}^{4+} : E_d(5/2) & = 229.8 \text{ eV}; E_d(3/2) = 233.1 \text{ eV} \\
\text{Mo}^{5+} : E_d(5/2) & = 231.1 \text{ eV}; E_d(3/2) = 234.3 \text{ eV} \\
\text{Mo}^{6+} : E_d(5/2) & = 232.8 \text{ eV}; E_d(3/2) = 235.9 \text{ eV}
\end{align*}
\]

The thickness of each film sample was measured with a digital vernier caliper (Keiba, Japan). Measurements were taken at four different places on the film and an average value were calculated.

3. Results and discussion

3.1. Production of BC, preparation of BCNCs and their properties

For BC production. \(K. \text{ xylinus}\) or \(G. \text{ xylinus}\) strains is the original microbial producer of BCNCs and has become more interesting in the model system for the study and research of biosynthetic mechanisms of BNC in bacteria (Keshk, 2014), because it produces a nearly nanofibrillar film with a gelatinous layer on the opposite side and a denser diagonal surface (Cai & Kim, 2010; Kurosumi, Sasaki, Yamashita, & Nakamura, 2009). For this study, \(K. \text{ xylinus}\) strain (TISTR No. 975) was cultured on agar, introduced into 2 L of glucose yeast extract nutrient broth (GYNB), and incubated at 30°C for 7 days under static conditions. The average yield of freeze-dried BC produced by fermenting with glucose as carbon source was 2.25 g/L of culture. The physical appearance of BC pellet is white-transparent single layer with dense and smooth surface (Fig. 1a–b) and is thus described by a highly crystalline linear polymer of glucose synthesized, absent of hemicellulose, pectin, lignin, or any other compound found in the plant-derived cellulose (Fu et al., 2013).

BCNCs are the crystalline domains hydrolyzed through acid hydrolysis, this method starts with the random cleavage of amorphous phases of very long cellulose micro-fibrils eventually giving raise to several fractions of BC hydrolysis products (George et al., 2011). They are quite rigid, several rod-like particles with an average length of up to hundreds of nanometers (Tang et al., 2017) and width of several nanometers (10–50 nm). For preparation of BCNCs, HCl and H\(_2\)SO\(_4\) were...
used to obtain BCNCs. Six acid hydrolysis conditions with time-varying were used to evaluate the influence of the acid types and reaction time on BCNCs preparation. H$_2$SO$_4$ produces a highly stable colloidal suspension (Fig. 1d) because of the high negative surface charge contributed by sulfonation of the CNCs surface. In contrast, when HCl is used (Fig. 1c) low-density surface charges is produced on the CNCs with limited nanocrystal dispersibility, which tends to contribute sediment in aqueous suspensions.

Some studies describe the companion use of H$_2$SO$_4$ and HCl to generate stable and thermally resistant CNCs suspensions (Corrêa et al., 2010). CNCs with different physical and mechanical properties can be yielded by the acid hydrolysis reaction conditions, which depend on the concentration and type of acid, reaction time, and temperature. The abundant thick bundles of BC fibers, which corresponded to the FE-SEM images and the TEM images (Figs. 2a and 3a). In contrast with HCl-hydrolyzed BC for 6 h (Figs. 2b and 3b) and H$_2$SO$_4$-hydrolyzed BC for 3 h (Figs. 2c and 3c) that show an increase of single fibers. The integrated structure of BC fiber bundles was broken into separated fibers and many small fiber bundles by penetrating of hydrogen ions (H$^+$) into amorphous cellulose molecules promoting cleavage of covalent or glycosidic linkage/bonds, therefore releasing single crystallites (Vasconcelos et al., 2017).

3.1.1. Z-discriminant function, $I_α/I_β$ ratio, crystallinity, and crystallite size

The XRD patterns of pristine BC and all acid-hydrolyzed BCNCs showed three 2θ diffraction peaks at around 15°, 17°, and 22° for cellulose I polymorphs (both $I_α$ and $I_β$ allomorphs), which are normally ascribed to crystallographic planes of 110, 010, 100 for $I_α$ and 200, 100, 1–10 for $I_β$, respectively (Wada et al., 2001). To analyze and classify all cellulose samples that were the algal-bacterial type ($I_α$-rich, $Z > 0$) or the cotton-ramie type ($I_β$-dominant, $Z < 0$) (Wada et al., 2001), the Z-values (Table 1) were calculated by substituting the $d_1$ and $d_2$ values listed in Table S2 into the Eq. (7) and plotted in Fig. S1. The intensity of the $d_1$ is higher than the $d_2$, which is a distinctive characteristic of typical cellulose that dominate mostly $I_α$ phase (Lee, Gu, Kafle, Catchmark, & Kim, 2015). All samples were small differences in d-spacing by the acid hydrolysis and classified as $I_α$-rich type (Z-value > 0). Otherwise, the relative content of $I_α/I_β$ were calculated in Table S2. Comparing the $I_α/I_β$ ratios of pristine BC (0.87/0.13), BCNCs-3 (0.83/0.17), and BCNCs-6 (0.9/0.1), the highest of $I_α/I_β$ ratio was found in H$_2$SO$_4$-hydrolyzed BCNCs (BCNCs-6) and the lowest of $I_α/I_β$ ratio was found in HCl-hydrolyzed BCNCs (BCNCs-3). The differences notwithstanding, it was observed that the choice of acid hydrolysis conditions slightly affect the $I_α/I_β$ ratio of bacterial cellulose. Nevertheless, the effect is not significant. Crystallinity is a main factor that significantly influences the physical, structural and mechanical properties of materials. Therefore, the XRD patterns obtained from HCl-hydrolyzed BC samples with hydrolysis time-varying at 4, 5, and 6 h (BCNCs-1 to 3) (Fig. 4a), XRD patterns obtained from H$_2$SO$_4$-hydrolyzed BC samples with hydrolysis time-varying at 1, 2, and 3 h (BCNCs-4 to 6) (Fig. 4b), and XRD patterns obtained from pristine BC, BCNCs-3 and BCNCs-6 (Fig. 4c) were used to determine the percent crystallinity (Cr (%)) and the crystallite size ($L_{hkl}$) of pristine BC and all acid-hydrolyzed BCNCs. The results indicated that all BCNCs had a Cr (%) (85.4–90.2%) greater than pristine BC (81.1%) (Table 1). The higher in crystallinity after acid hydrolysis reaction was because of a rapid and continuous decrease of the amorphous content, as this amorphous phase is to a higher degree accessible to acid penetration. Crystallinity
is a main factor that significantly influences the physical, structural and mechanical properties of materials. Therefore, the XRD patterns obtained from HCl-hydrolyzed BC samples with hydrolysis time-varying at 4, 5, and 6 h (BCNCs-1 to 3) (Fig. 4a), XRD patterns obtained from pristine BC, BCNCs-3 and BCNCs-6 (Fig. 4c) were used to determine the percent crystallinity (Cr (%)) and the crystallite size (Lhkl) of pristine BC and all acid-hydrolyzed BCNCs. The results indicated that all BCNCs had a Cr (%) (85.4–90.2%) greater than pristine BC (81.1.%) (Table 1). The higher in crystallinity after acid hydrolysis reaction was because of a rapid and continuous decrease of the amorphous content, as this amorphous phase is to a higher degree accessible to acid penetration. The comparison of the Cr (%) of BCNCs-3 and BCNCs-6 (which differed in acid types and the reaction time), significant difference was detected. For BCNCs-6 (involving a higher reaction time), a decrease in the Cr (%) and the crystallite size was observed, possibly due to the harsh hydrolysis conditions, resulting in a probably change in the orientation of the BC main chains. Contrarily, BCNCs-3 showed a higher Cr (%) than the BCNCs obtained after acid hydrolysis H2SO4. From this, it was evident that H2SO4 involved in the integration structure of BC fiber bundles significantly influence the cleavage of covalent or glycosidic linkage/bonds, therefore generating smaller single crystallite size with higher amorphous contents. Additionally, the crystallite size (Lhkl) of pristine BC and all acid-hydrolyzed BCNCs were calculated from the XRD spectra by using the Scherrer equation (Singhsa et al., 2018a; Vasconcelos et al., 2017) (Table 1). The pristine BC showed the larger Lhkl (6.9nm) than their nanocrystals. Compared with the BCNC-3 and BCNC-6 conditions, the nanocrystals obtained under time-varying hydrolysis conditions showed slight differences in the Lhkl values. The Lhkl was slightly decreased with increasing acid hydrolysis reaction time. The reduction in the Lhkl of acid-hydrolyzed BCNCs purposed that acid hydrolysis is tend to reducing the crystalline part of the original materials (Castro et al., 2011; Singhsa et al., 2018a). Thus, we conclude that the Lhkl of H2SO4-hydrolyzed BCNCs with the highest acid hydrolysis reaction time (BCNCs-6) were the smallest (4.6nm) compared to other acid hydrolysis conditions; these results could confirm the high surface area of BCNCs are successfully developed by H2SO4 acid hydrolysis for increasing its dispersibility into other matrix materials as an excellent additive material, while maintaining the unique properties of pristine BC.

### 3.1.2. Hydrodynamic sizes and ζ-potential

The physicochemical characterization of pristine BC and BCNCs obtained through acid hydrolysis under different conditions (Table 1). For H2SO4 acid hydrolysis reaction, H2SO4 has the ability to esterify the hydroxyl (OH−) groups of cellulose with sulfate groups (−OSO3−) to offer an acid half-ester or the so-called cellulose sulfate (sulfated cellulose) (Singhsa et al., 2018a), this is cause to generate negatively charged surface nanocrystals. Accordingly, the strong acid hydrolytic activity of H2SO4, the BCNCs prepared by H2SO4 acid hydrolysis for increasing its dispersibility into other matrix materials as an excellent additive material, while maintaining the unique properties of pristine BC.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>XRD</th>
<th>FT-IR</th>
<th>Zetasizer</th>
<th>BET</th>
<th>Nanosizer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crystal size (nm)</td>
<td>Crystallinity (%)</td>
<td>Z-values</td>
<td>Cellulose Iα content (%)</td>
<td>ζ-potential (mV)</td>
</tr>
<tr>
<td>Pristine BC</td>
<td>6.9</td>
<td>81.1</td>
<td>+8.47</td>
<td>90.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>BCNCs-1</td>
<td>5.9</td>
<td>86.4</td>
<td>+8.63</td>
<td>91.0</td>
<td>n.d.</td>
</tr>
<tr>
<td>BCNCs-2</td>
<td>5.8</td>
<td>88.7</td>
<td>+7.49</td>
<td>91.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>BCNCs-3</td>
<td>5.3</td>
<td>90.2</td>
<td>+7.45</td>
<td>91.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>BCNCs-4</td>
<td>4.9</td>
<td>85.4</td>
<td>+8.59</td>
<td>91.8</td>
<td>n.d.</td>
</tr>
<tr>
<td>BCNCs-5</td>
<td>4.7</td>
<td>86.7</td>
<td>+8.99</td>
<td>91.0</td>
<td>n.d.</td>
</tr>
<tr>
<td>BCNCs-6</td>
<td>4.6</td>
<td>88.5</td>
<td>+9.17</td>
<td>92.3</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d.: not determine.

**Fig. 4.** XRD patterns obtained from HCl-hydrolyzed BC with hydrolysis time-varying at 4, 5, and 6 h (BCNCs-1 to 3) (a), XRD patterns obtained from H2SO4-hydrolyzed BC with hydrolysis time-varying at 1, 2, and 3 h (BCNCs-4 to 6) (b), XRD patterns obtained from non-hydrolyzed BC, BCNCs-3, and BCNCs-6 (c).
be received. In contrast, HCl has milder acid-hydrolysis reaction and basically promotes cellulose crystallites with native crystalline structures that have no evident of sulfate groups, resulting in the production of neutral nanocrystals. Therefore, the hydrodynamic sizes of the HCl-hydrolyzed BCNCs were larger, 1153, 1142, and 1122 d.nm for BCNCs-1, BCNCs-2, and BCNCs-3, respectively.

The zeta potential is commonly applied to indicate the presence of surface charges on any material that can be correlated to the zeta potential of the particles. In addition, zeta potential is important for a superior understanding on the character of the particle stability, and it has also been well-known that suspension particles with higher absolute values of the zeta potential have a tendency to be less aggregated by reason of high electrical repulsion among the particles (Elimelech, Nagai, Ko, & Ryan, 2000; Vasconcelos et al., 2017). Zeta potential values with a modulus with higher than 30mV reflect good stability of a system, while values with a modulus with lower than 30mV reflect the systems exhibiting only electrostatic attraction (Elimelech, Nagai, Ko, & Ryan, 2000; Vasconcelos et al., 2017). Consequently, the remarkable hydrodynamic sizes and zeta-potential values of the H2SO4-hydrolyzed BCNCs were found in H2SO4− hydrolyzed BCNCs, about -21.5, -21.9, and -22.6 mV for BCNCs-1, BCNCs-2, and BCNCs-3, respectively. For HCl-hydrolyzed BCNCs, the presence of lower negative charges could be the reason of a significantly increased oxidation of the BC main chains by reactive oxygen species throughout BC acid hydrolysis, causing negatively charged functional groups, such as for example, carboxylic groups on the surface of nanocrystals (Winter et al., 2010). Consequently, the remarkable hydrodynamic sizes and zeta-potential values of the H2SO4− hydrolyzed BCNCs was successfully developed for increasing its dispersibility on alginate-based film while maintaining the unique properties of pristine BC, thus preventing their aggregation, and also for increasing AgNPs dispersibility, leading AgNPs to be more possibility of access to H2S gas.

### 3.1.3. Total specific surface area and micro-pore volume

The BET-adsorption isotherm curve of pristine BC, HCl-hydrolyzed BCNCs (BCNCs-3), and H2SO4− hydrolyzed BCNCs (BCNCs-6) were characterized (Fig. S2) in order to identify the total specific surface area, \( A_t \) (m²/g) and micro-pore volume, \( V_m \) (cm³(STP)/g) (Table 1). As a result of the strong acid hydrolytic activity, the BCNCs prepared by HCl and H2SO4 hydrolysis, we should expect an enlarged total specific surface area. Comparing the \( A_t \) of pristine BC (3.1 m²/g), BCNCs-3 (6.0 m²/g), and BCNCs-6 (6.3 m²/g), the highest of \( A_t \) was found in H2SO4− hydrolyzed BCNCs (BCNCs-6) and the lowest of \( A_t \) was found in pristine BC. The results of this measurement are closely linked with those of FT-IR analysis. However, there was no evident IR peak of the 807 cm⁻¹ band for C–O–C glycoside bonds (Vasconcelos et al., 2017), and 700–800 cm⁻¹ (H-bonding vibrations) (Zygmunt-Chargot, Cybulska, & Zdunek, 2011).

### 3.2. Preparation of BCNCs based surface-loaded AgNPs and their properties

Accordingly, the previous results in Table 1, found that suitable condition of acid hydrolysis reaction for preparing BCNCs with H2SO4 hydrolyzed BCNCs at 45°C for 3 h (BCNCs-6) when compared to those with different conditions, resulted in the smallest hydrodynamic sizes (854 d.nm) the smallest crystallite sizes (4.6 nm), and the highest negative surface ζ-potential values (-34.3 mV); these results could confirm the high surface area and the high dispersibility by increasingly interacting with other materials.

#### 3.2.1. Formation of AgNPs on BCNCs-6

By the immersion of BCNCs-6 in silver ion Ag⁺ aqueous solutions using Ag(NO₃)₂ as Ag⁺ precursors, the high surface of the several single fibers with three-dimensional network structure of the BCNCs-6, as shown in Fig. 2c, could allow Ag⁺ ions to penetrate into the BCNCs-6 structure as long as the adsorption equilibrium is accomplished. According to, Ag⁺ ions is impregnated and loaded onto the stable adsorption site via electrostatic repulsive interaction between Ag⁺ ions and lone pair electrons of OH (hydroxyl) groups on the surface of BCNCs-6 nanofibers (Azizi, Ahmad, Mahdavi, & Abdulomohammadi, 2013; Janpetch et al., 2016), as shown in chemical Eqs. (9)–(12):

\[
4\text{Ag(NO}_3)_2 (s) + 2\text{H}_2\text{O} \rightarrow 4\text{Ag}^{+} + 4\text{HNO}_3 + \text{O}_2
\]

\[
\text{Ag}^{+} + \text{surface of BCNCs} \rightarrow \text{Ag}^{+} \text{BCNCs}
\]

\[
\text{Ag}^{+} \text{BCNCs + OH} \rightarrow \text{AgOH}... \text{BCNCs}
\]
After the stable absorption site via electrostatic repulsive interaction of Ag⁺ ions on BCNCs fibers, the Ag⁺ saturated-BCNCs were ion-reduced and transformed to silver nanoparticles (AgNPs) deposited on the surface of BCNCs fibers via the chemical reaction with OH⁻ from NH₄OH (Janpetch et al., 2016; Li, Shi, Zhong, & Yin, 1999; Yang, Zhu, Liu, Chen, & Ma, 2008), as shown in chemical Eq. (13)

\[
2\text{OH}^- + 2\text{Ag}^+ \rightarrow \text{AgO} + \text{AgNPs} \, . \, \text{BCNCs} + \text{H}_2\text{O} \quad (13)
\]

Consequently, morphological properties of the obtained dry BCNCs–AgNPs samples were analyzed.

3.2.2. Microstructural characteristics of BCNCs–AgNPs

Morphological analysis of the obtained BCNCs–AgNPs samples by TEM micrograph (Fig. 5), the average size and shape of the AgNPs were approximately in the range from 1 nm to 10 nm although some agglomeration was also present. The results from these micrographs could confirm the well-dispersed of AgNPs with the high surface area of BCNCs-6 by increasingly interacting surface between each other.

3.2.3. Elemental analysis of BCNCs–AgNPs

The elemental composition of BCNCs–AgNPs was determined by the energy-dispersive X-ray (EDX) analysis, the samples of BCNCs–AgNPs were coated with platinum (Pt) by ion sputtering. The EDX images in Fig. S5a and b were analyzed by X-ray dot mapping mode and the EDX images in Fig. S5c was taken by using element identification mode. In Fig. S5c, EDX images of BCNCs–AgNPs sporadically surface-loaded by AgNPs with which they closely interact to BCNCs-6 to form a physical adsorption. There are significant peaks of the Ag element, which belong to AgNPs is loaded on the surface of BCNC-6 with homogeneously dispersed, as shown in Fig. S5b. Thus, it is confirmed that the hybridization of AgNPs and BCNCs by using the chemical reducing reagent under ultrasonic treatment solution could accomplish the hybridization by physical adsorption of AgNPs on BCNCs’s surface. They also exhibit the rather high peak of the C and O element, which are a general component of BCNCs and other cellulose material.

3.3. Preparation of hybrid BCNCs–AgNPs/alginate–MoO₃NPs film and their properties

3.3.1. Formation of hybrid BCNCs–AgNPs/alginate–MoO₃NPs film

The demonstration of the hybrid material of BCNCs–AgNPs/alginate–MoO₃NPs prepared by physical adsorption techniques and its film form are shown in Fig. 6a and b. The hybrid of the BCNCs–AgNPs/alginate–MoO₃NPs in the form of solution and film can be both in Mo(V)O₃ (Mo⁵⁺) and Mo(VI)O₃ (Mo⁶⁺) characterized by x-ray photoelectron spectroscopy (XPS) technique.

The preparation of Mo(VI)O₃NPs solution with ultrapure water Eq. (14), Mo⁵⁺●H₂O and Mo⁶⁺●H₂O were occurred without separation of the new phase. The two water molecules in Mo⁵⁺●H₂O and Mo⁶⁺●H₂O
are different bonded types, depending on the oxidation state of Mo. For Mo\(^{5+}\) \(\cdot\) H\(_2\)O, in addition of oxygens, is coordinated to Mo atoms in one layer to oxygen ions in the next layer, and for the Mo\(^{6+}\) \(\cdot\) H\(_2\)O is held by hydrogen bonding in between the layers of MoO\(_3\) (Günter, 1972). By the immersion of sodium alginate in the Mo(VI)O\(_3\)NPs solution, the carboxylate side groups with several of the negative charges of alginate Eq. (Eq. 15), is highly reactive to cations of Mo\(^{5+}\) to form Mo\(^{5+}\)Alginate, and also to electrostatic repulsive interaction with Mo\(^{6+}\) to form Mo\(^{6+}\)–Algin-ate– Eq. (Eq. 16).

\[
\text{Mo(VI)O_3NPs (s) + 2H_2O \rightarrow Mo^{5+} \cdot H_2O + Mo^{6+} \cdot H_2O (14)}
\]

Sodium alginate (s) + H\(_2\)O \(\rightarrow\) Na\(^+\) + Alginate-

\[
\text{Mo}^{5+}\cdot\text{H}_2\text{O} + \text{Mo}^{6+}\cdot\text{H}_2\text{O} + \text{Alginate} \rightarrow \text{Mo}^{5+}\text{Alginate} + \text{Mo}^{6+}\text{--Alginate}-(15)
\]

By hydriding the dried BCNCs–AgNPs to alginate–MoO\(_3\)NPs solution, there are two different types of surface negative charges on the surface of BCNCs–AgNPs: (i) from sulfate groups (SO\(_3\)-); and (ii) from hydroxyl groups (OH-), and thus causing different interactions with Mo and alginate (Eqs. (17)-(18)).

\[
\text{Mo}^{5+}\text{Alginate} + \text{Mo}^{6+}\text{--Alginate} - \text{BCNCs–AgNPs (SO}_3^-) \rightarrow \text{Mo}^{5+}\text{Alginate} + \text{Mo}^{6+}\text{--Alginate} - \text{BCNCs–AgNPs (SO}_3^-) + \text{Mo}^{6+}\text{--Alginate} - \text{BCNCs–AgNPs (OH}-) (17)
\]

\[
\text{Mo}^{5+}\text{Alginate} + \text{Mo}^{6+}\text{--Alginate} - \text{BCNCs–AgNPs (OH}-) \rightarrow \text{Mo}^{5+}\text{Alginate} + \text{Mo}^{6+}\text{--Alginate} - \text{BCNCs–AgNPs (OH}-) + \text{Mo}^{6+}\text{--Alginate} - \text{BCNCs–AgNPs (OH}-) (18)
\]

Consequently, properties of the obtained hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film samples were analyzed and H\(_2\)S gas sensor ability was characterized using XPS with H\(_2\)S gas exposure experiment.

3.3.2. Physical appearances of the film

The hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs films were visually homogeneous with no air bubbles, wrinkles, snags, or brittle areas and were easily manipulatable and moderately flexible. The appearance of the film was not significantly affected by the addition of bacterial cellulose and metal nanoparticles at a 50 mM of MoO\(_3\) solution and 0.1 dry wt% of BCNCs–AgNPs except for the film color which was changed very little from its initial alginate film (transparent white color). The transparency of the film was evaluated visually and photographed (Fig. 6b), showing the development of a light greyish-white color, depending on the MoO\(_3\) content. The average thickness of the film measured by a micrometer was approximately 90\(\mu\)m.

3.3.3. Identification of alginate and MoO\(_3\)NPs

FTIR analysis of the BCNCs–6, BCNCs–6–AgNPs/alginate–MoO\(_3\)NPs film, and alginate film attempted to characterize the hybridization of BCNCs–6–AgNPs and alginate–MoO\(_3\)NPs by distinguishing the infrared (IR) bands and vibration shifts related to the BCNCs–alginate and MoO\(_3\)NPs–alginate interactions (Fig. S6). The characteristic peak of alginate polysaccharide vibration bands, such as the peak at 1602 cm\(^{-1}\) (\(\sim\)C\(\equiv\)O carboxyl bonds), 1424 and 1024 cm\(^{-1}\) in both alginate and BCNCs–alginate bands are assigned to (\(\sim\)COO and \(\sim\)C\(\equiv\)O carboxyl stretching bands), and 2930 cm\(^{-1}\) \((\sim\)CH aliphatic chain stretching vibrations), and 2930 cm\(^{-1}\) \((\sim\)CH aliphatic chain stretching vibrations). For the hybrid BCNCs–6–AgNPs/alginate–MoO\(_3\)NPs film, the Mo–O bonding vibration bands is clearly shown as a sharp band centered at about 950 cm\(^{-1}\), and characteristic peaks were present in the 953–957 cm\(^{-1}\) range, which can be determined to the stretching mode of the terminal Mo–O group (Ashsharouh, Majhoub, & Amini, 2009; Bhattacharya, Dinda, & Saha, 2015) and 865 cm\(^{-1}\) are assigned to the Mo–O–Mo stretching vibrations of MoO\(_3\) (Bhattacharya et al., 2015). Some reports showed a high peak intensity of terminal Mo–O bonds (993 cm\(^{-1}\) and 817 cm\(^{-1}\)) presents a large oxygen vacancy (Bhattacharya et al., 2015). Some differences can be observed after MoO\(_3\)NPs addition into the alginate matrix. Consequently, occurrence of the peak with a sharp band centered at about 950 cm\(^{-1}\) and 865 cm\(^{-1}\) were observed as the MoO\(_3\)NPs was incorporated into the alginate matrix. These results can represent the existence of the ionic bonding and electrostatic repulsive interaction between the alginate and MoO\(_3\)NPs. MoO\(_3\)NPs in the form of Mo(V)O\(_3\) (Mo\(^{5+}\)) are formed an ionic bonded with carboxylic acid groups (\(\sim\)COO–) on the alginate chain, and MoO\(_3\)NPs in the form of Mo(VI)O\(_3\) (Mo\(^{6+}\)) are physically adsorbed on both in the alginate chain and sulfate groups (\(\sim\)OSO\(_3^-\)) on the BCNCs’s surface by electrostatic interaction.

3.3.4. Elemental analysis of BCNCs–AgNPs/alginate–MoO\(_3\)NPs film

The elemental composition of the hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film was determined by EDX analysis, the film sample was coated with platinum (Pt) by ion sputtering. The EDX images in Fig. S7a-c were analyzed by X-ray dot mapping mode and the EDX images in Fig. S7d was taken by using element identification mode. In Fig. S7d, EDX images of the hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film based on the alginate matrix, sporadically hybridized by MoO\(_3\)NPs and BCNCs–AgNPs with which they closely interact to alginate to form a physical adsorption. There are significant peaks of the Ag element, which belong to AgNPs are loaded on the surface of BCNCs–6 with homogeneously dispersed, as shown in Fig. S7b. Besides, the significant peaks of the Mo element, which belong to MoO\(_3\)NPs are loaded on the alginate with homogeneously dispersed, as shown in Fig. S7c. Thus, it is confirmed that the hybridization of: (i) alginate and MoO\(_3\)NPs by using ultrasonic treatment solution could accomplish the hybridization by physical adsorption by covalent and ionic bonds of MoO\(_3\)NPs with carboxylic acid groups (\(\sim\)COO–) on the alginate chain; and (ii) BCNCs–AgNPs by using the chemical reducing reagent under ultrasonic treatment solution could accomplish the hybridization by physical adsorption of AgNPs on BCNCs’s surface. They also exhibit the rather high peak of the C and O element, which are a general component of alginate polysaccharide, BCNCs and other cellulosic material.

3.4. H\(_2\)S gas exposure experiment

3.4.1. H\(_2\)S gas sensor ability of hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film

For the H\(_2\)S gas exposure experiment, the demonstration of the in-house H\(_2\)S gas preparation and H\(_2\)S gas exposure experiment of hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film is shown in Fig. S8. The concentration of H\(_2\)S gas was calculated to be approximately 1400 ppm in the tightly septum-capped glass bottle and then was diluted 100 times by displaced 5 mL of the H\(_2\)S gas using syringe plunger to each of the film sample bottles with the same bottle volume. Finally, each of the film sample bottles was reached the H\(_2\)S gas in concentration of approximately 14 ppm and leave them with gas exposure time-varying for 5 min, 1 h, and 24 h. The fast H\(_2\)S gas sensor of the hybrid BCNCs–AgNPs/alginate–MoO\(_3\)NPs film would be highly useful for a low concentration of H\(_2\)S at 14 ppm, in which increase of the Mo sub-oxide color intensity can represent the existence of the ionic bonding and electrostatic repulsive interaction between the alginate and MoO\(_3\)NPs. MoO\(_3\)NPs in the form of Mo(V)O\(_3\) (Mo\(^{5+}\)) are formed an ionic bonded with carboxylic acid groups (\(\sim\)COO–) on the alginate chain, and MoO\(_3\)NPs in the form of Mo(VI)O\(_3\) (Mo\(^{6+}\)) are physically adsorbed on both in the alginate chain and sulfate groups (\(\sim\)OSO\(_3^-\)) on the BCNCs’s surface by electrostatic interaction.
from transparent light-grey to opaque dark brown-black can be assigned to the conversion of Ag to Ag₂S to produce atomic H, and then the Mo to from MoO₃NPs are readily reduced to a colored sub-oxide by atomic H.

3.4.2. Mechanism of H₂S sensing

Pristine MoO₃ consists of Mo(VI)O₃(Mo⁶⁺) forming the conduction bands and O²⁻ forming the valence bands (Borgschulte et al., 2017) can be assigned to Mo⁶⁺ 3d⁵/₂ and Mo⁶⁺ 3d³/₂, respectively (Gong & Haur, 2017; Ji et al., 2013). The first reaction is believed to occur at the HS⁻ adsorbed on the AgNPs of the well-dispersed BCNCs–AgNPs to readily form Ag₂S and promptly generated H₂. Next, the intercalation of H₂ into MoO₃ by the vicinity of H₂ to oxygen without alteration of the structural motif apart from few increase of the cell volume (Borgschulte et al., 2017), and water is formed at longer gas exposure time. The dissociation of hydrogen molecule from the reaction of AgNPs and H₂S gas on oxide surfaces of MoO₃NPs in the film, which decomposes into MoO₂⁺⁵/₂ and H₂O by the fast decrease of Mo oxidation state from Mo⁶⁺ into Mo⁵⁺, slowly forms Mo⁴⁺ states, forms a very low-intensity MoS₂⁴⁺ at exposure time of 1h, and forms a low-intensity MoS₂⁴⁺ at exposure time of 24h. Besides, the increase of AgNPs dispersibility by well-dispersed BCNCs–AgNPs in alginate–MoO₃ matrix phase, leading AgNPs to more possibility of access to H₂S gas. The Mo(IV)S₂(Mo⁴⁺) is considerably increased due to the loss of O₂ depending on gas exposure time of sampling. These conversion can be described by the chemical reactions as shown in chemical Eqs. (19)–(22):

8Ag + 4HS⁻ → 4Ag₂S + 2H₂ + 4e⁻ \hspace{1cm} (19)

Mo(V)O₃ + H₂ → Mo(V)O₃ + H₂ + e⁻ \hspace{1cm} (20)

Mo(V)O₃ + H₂ → Mo(IV)O₂ + H₂O \hspace{1cm} (21)

Mo(IV)O₂ + 2HS⁻ → Mo(IV)S₂ + H₂ + O₂²⁻ \hspace{1cm} (22)

The rate of reaction increases with the increase in gas exposure time. These mechanism fully support the H₂S gas sensor from the hybrid BCNCs–AgNPs/alginate–MoO₃NPs film by this gas exposure experiment.

3.4.3. XPS measurement

H₂S gas sensing characteristics of the films were examined from XPS spectrogram. A high-resolution XPS measurement was carried out in order to examine the surface chemical composition and the oxidation states of Mo. Fig. 8 shows the XPS spectra of the hybrid BCNCs–AgNPs/alginate–MoO₃NPs films in order to examine the oxidation states of the Mo 3d, 5d, and 2s region for Mo; before exposing to H₂S gas (a), after exposing to 5 mL of H₂S gas for 5 min (b), 1 h (c), and 24 h (d).

![Fig. 8. XPS spectra of the BCNCs–AgNPs/alginate–MoO₃NPs films in order to examine the valence states of the Mo 3d, 5d, and 2s region for Mo; before exposing to H₂S gas (a), after exposing to 5 mL of H₂S gas for 5 min (b), 1 h (c), and 24 h (d).](image-url)
5 min, 1 h, and 24 h, respectively as shown in Table S4.

The ratio is determined based on: (i) the oxidation state or valence state of MoO$_2$ after losing the O molecule by H$_2$ intercalation (Mo(IV) O$_2$(Mo(IV))); and (ii) the oxidation state of pristine MoO$_2$ (Mo(VI) O$_2$(Mo(VI))). Thus, the conversion of Mo(VI) to Mo(IV) is depend on gas exposure time with the slower increase of the ratio value due to some limitation of Mo oxide surfaces for H$_2$ intercalation.

4. Conclusion

In this work, BC was successfully produced by bacterial strain of K. xylinus under static culture with average yield was 2.25 g/L of culture. The acid conditions profoundly influenced the physical properties of nanocrystals including the morphology, hydrodynamic size, ζ-potential, crystallite size, and CI. Accordingly, BCNCs was successfully achieved by acid hydrolysis under various acid conditions, namely, HCl, H$_2$SO$_4$ with time-varying. Resulting in H$_2$SO$_4$ acid-hydrolyzed BCNCs (BCNCs-6) were evaluated for their highest surface area (6.3 m$^2$/g) for interaction with metallic nanoparticles with the smallest crystallite size (4.6 nm) and hydrodynamic size (854.1 nm) with the most remarkable ζ-potential value (-34.3 eV) for negative surface charge. Thus, BCNCs-6 was selected for the study of BCNCs surface-loaded AgNPs and then hybridized with alginatr-MoO$_3$NPs by physical adsorption under ultrasonic treatment to get film structure for H$_2$S gas sensor ability. Thus, the high surface area of BCNCs enables its dispersibility on alginate-based film while maintaining the unique properties of pristine BC, thus preventing their aggregation, and also for increasing AgNPs dispersibility, leading AgNPs to be more possibility of access to H$_2$S gas. H$_2$S gas with concentration 14 ppm, have been utilized for the measurements. The hybrid film of BCNCs-AgNPs/alginate–MoO$_3$NPs was successfully developed for H$_2$S gas sensor by reason of a change in Mo oxidation state and the reduction mechanism by atomic hydrogen intercalating of MoO$_2$NPs on the film. The Mo oxidation state on the film under H$_2$S gas exposure can be Mo(VI)$\rightarrow$Mo(V)+, Mo(V)$\rightarrow$Mo(IV)+, and Mo(VI)O$_2$ (Mo(VI)+) examined from XPS spectrogram. The film color are changed from transparent light greyish-white to opaque dark brown-black indicated the dissociation of hydrogen molecule from the reaction of AgNPs and H$_2$S gas on oxide surfaces, which decomposes into MoO$_2$ + 1/2 H$_2$O by the fast decrease of Mo oxidation state from Mo(VI) into Mo(IV), slowly forms Mo(IV)+ states, forms a very low-intensity Mo(IV)+ at exposure time of 1 h, and forms a low-intensity Mo(IV)+ at exposure time of 24 h. The color intensity is gradually increased according to the increase of exposure time. Consequently, this study could provide useful information about the preparation of rod-like BCNCs from BC produced by K. xylinus strain under static culture, surface-loaded with metal nanoparticles, and hybrid with alginatr-MoO$_2$ to reach H$_2$S gas sensor ability, in addition to rendering the properties of nanocrystals in accordance with the desired applications.

Acknowledgements

This research was supported by the Petroleum and Petrochemical College, Chulalongkorn University. One of the authors, P.S., would like to acknowledge the scholarship from the 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship and the 90th Anniversary of Chulalongkorn University Scholarship (Ratchadaphiseksomphot Endowment Fund) as well as supported by Graduate School of Chulalongkorn University. Part of research grant was supported by Center of Excellence on Petrochemicals and Materials Technology, Chulalongkorn University.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.carbpol.2019.115566.
molybdenum trioxide with promoted performance as an anode material for lithium-ion batteries. *Journal of Materials Chemistry A*, 2.


