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Properties and aerogel applications of a marine algal origin biocellulose produced by the immobilized *Gluconacetobacter xylinus* ATCC 10245

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ABSTRACT

Algae are known to produce biomass faster as compared with lignocellulosic biomass. BC was produced by using reducing sugar from algae as a cheaper carbon source. The effect of immobilization on the production of biocellulose(BC) by *Gluconacetobacter xylinus* ATCC 10245 was studied. The BC production of all entrapped cultures within different gel materials was lower than that of free cell cultures but the entrapped *G. xylinus* cells in alginate beads recorded the highest BC productivity (12.5 g/l). The adsorption of *G. xylinus* cells on luffa pulp (LP) and ceramics particles (CP) gave the highest BC production (14.5 g/l), which represents increasing of 1.14 and 1.05- fold, respectively from the free cells. This attempt clarified that LP was the best immobilizing support as it gave the highest BC production. The effect of repeated reuse of LP was confirmed. Different physical properties of BC including; pH, water absorption capacity, dissolving in several solvents, and chemicals were determined. Observation of BC through SEM analysis showed the fine cellulose ribbons (fibrils). The crystallinity for the BC produced from X-ray diffraction (XRD) spectra was 84%. Upon the applicable level, the BC aerogel was formed to be applied in rising up the water and oil absorption capability by developing its hydrophilic properties. Data introduced strong affinities to the motor, olive, and cooking oils with high oil uptake contents of 7.5, 5.2, and 3.7 gg^{-1}, respectively. Moreover, the BC aerogel exhibited potential adsorption for different heavy metal ions Pb^{2+}, Cd^{2+}, and Cu^{2+}. The highest capability of Cd^{2+} removal was 95.7% at pH 6. The biodegrability of our produced BC and then its safety was proven.

INTRODUCTION

In recent years, interest in algae is continuously increasing since algae have potential to meet global demand in addition to that microalgae have various advantages over other agricultural raw material (Szulczyk, 2010). Most significant is that they can be grown anywhere and under any weather conditions because algae have variety of species

with different properties and adaptations ability. It can grow in open ponds, sea water, fresh water, deserts, arid lands, etc (Goyat, 2012). Algae are known to produce biomass faster and on reduced land surface as compared with lignocellulosic biomass (Lee, 2013). The cell wall of algae consists of polysaccharides that can be hydrolyzed to produce sugar. Therefore, algae can be used as a carbon source in fermentation processes. All these characteristics make algae a promising alternative for agricultural residues (Goyat, 2012). BC was produced by using reducing sugar from algae as a cheaper carbon source (Beliah *et al*., 2020). Biocellulose (BC) is produced by an acetic acid-producing bacterium; *Gluconacetobacter xylinus* with a diameter of about 1/100 of that of plant cellulose (Esa *et al*., 2014). Currently, different kinds of microbial immobilization have found wide applications in many fields (biotechnology, pharmaceutical, environmental, food, and biosensor industries). By immobilization, microorganisms may retain on a carrier can be used in continuous and semi-continuous production processes allowing for significant cost decrease, as the biocatalyst does not need to be refilled (Mrudula and Shyam, 2012).

The carriers are classified as inorganic material (such as: zeolite, clay, anthracite, porous glass and ceramics) and organic polymers (such as: alginate, carrageenan, agar, collagen, gelatin, cellulose, polyacrylamide, polyester, polystyrene, and polyurethane). The most commonly used polymers are the natural polymers alginate and carrageenan but these natural polymers are less stable in wastewater than synthetic polymers (Stolarzewicz *et al*., 2011).

However, the entrapment is the most method extensively studied in cell immobilization. It is also an irreversible process, where immobilized cells are entrapped in a support matrix or inside fibers. This technique creates a protective barrier around the immobilized microbes, ensuring their prolonged viability during not only processing but also storage in polymers (Górecka and Jastrzębska, 2011).

On the other side, the BC has many properties such as high degree of crystallinity, high purity, high density, high water binding capacity, good shape retention, and higher surface area as compared to the native cellulose. It can be used in various areas including paper, textile industry, pharmaceutical, food, waste treatment, mining, and refinery (Shah and Brown, 2005; Czaja *et al*., 2006).

Particularly, aerogel is officially defined as gel comprised of microporous solid, in which the dispersed phase is a gas (Alemán *et al*., 2006). Because of the abundant choices of raw materials and the highly porous structure with mechanical strength, aerogels possessing significant features have been employed for many versatile applications (Tan *et al*., 2001). Network gels were formed by cross-linking cellulose acetate and cellulose acetate butyrate with tolylene 2,4-diisocyanate. However, they claimed that cellulose aerogels were not adequate to form carbon aerogels due to their rapid decomposition during pyrolysis. Ironically, a type of ultralight and flexible carbon aerogels were produced by pyrolyzing bacterial cellulose foams (Wu *et al*., 2014). In addition, cellulose based aerogels have also been used as adsorbents to remove pollutants from aqueous systems (Zhao *et al*., 2015).

Therefore, the current study aimed to investigate the influence of immobilization on the production of BC by *Gluconacetobacter xylinus* ATCC 10245 and its different properties. As well as, it conducted to some potential applications by using the BC aerogel prepared from this bacterium.

MATERIALS AND METHODS

Bacterial strain used for BC production and culture media

Bacterial strain used throughout this study was *Gluconacetobacter xylinus* ATCC 10245, which was obtained from Microbial Culture Collection, Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

The bacterial strain was maintained on glucose yeast calcium carbonate (GYC) agar slants, stored at 4°C and subcultured every two weeks to maintain good viability and stability for the BC production.

From previous work regarding optimization of the BC production by *Gluconacetobacter xylinus* ATCC 10245 (Beliah, 2017); reducing sugar was extracted from algae; *Posidonia oceanic* treated with 1% H_2SO_4 as a marine-origin carbon source. Consequently, the optimized medium contained (g/l): reducing sugar, 30; yeast extract, 7; peptone, 7; dibasic sodium phosphate (buffer), 3.7; citric acid, 0.2 under static condition within incubation period of 10 days.

Saccharification process using submerged fermentation

The submerged fermentation process was carried by medium containing pretreated substrate. A volume of 100 ml of aged filtered sea water amended with 3 g from pretreated substrate as sole carbon source and inoculated with a volume of 100 ml of sterile modified Czapek's-Dox broth medium was added to 3g from pretreated substrate as sole carbon source and inoculated with 3 ml freshly prepared inoculum. The flasks were loaded on a rotary shaker incubator at a speed of 120 ± 2 rpm at 28°C ± 2°C for 6 days. After incubation, the production broths were centrifuged at 5000 rpm for 15 min. The supernatants were collected for determining the reducing sugars concentration and enzymatic activity (El-Naggar *et al*., 2014).

Estimation of reducing sugars

The amount of reducing sugars was estimated by dinitrosalicylic acid method (Miller, 1959). 0.5 ml of sample was drawn from every sample into test tubes. The volume was made up to 3 ml using distilled water. Dinitrocsalicylic acid (DNSA) reagent of 3 ml were added to each sample and mixed well. The reagent blank containing 3 ml of distilled water and 3 ml of DNSA reagent was also prepared. Similarly, standards were also included whose glucose concentration ranged from 200 to 1000 µg. All tubes were kept on boiling water bath for 15 min. After this, one.ml of 40% Rochelle salt solution was added when the reaction mixture was still warm. Then the tubes were cooled. The samples were read at at the wave length 510 nm. Glucose was used as the standard for this analysis.

Immobilization of *G. xylinus* ATCC 10245 cells by entrapment

Gluconacetobacter xylinus ATCC 10245 cells were entrapped within four organic matreces (alginate, carragennen, agar, and agarose). In the frist matrix, the cells were entrapped in 3% calcium alginate gel as described by Sankaralingam *et al*. (2012). Secondly, the bacterial cells were entrapped in 3% k-carragennen gel as described by

Wada *et al*. (1980). To entrap the cells in the agar and agarose, the gel was prepared by dissolving 0.9 g of agar or agarose in 27 ml distilled water. After sterilization of the solution (by autoclaving at 121^{o}C for 20 min), three ml of bacterial cells were added to the gel solution and 10 ml were poured into sterilized petri dish. After solidification, the gel was cutted with a sterile cutter into small cubes, then transferred into 30 ml of sterilized medium and incubated at 30^{o}C for 15 days under static conditions.

Immobilization of *G. xylinus* ATCC 10245 cells by adsorption

One ml of bacterial cells was added to 30 ml of sterilized culture medium and about 10 cubes of synthetic sponge cubes (SSC) or luffa pulb (LP) or clay and pumice particles in 100 ml-Erlenmeyer flasks. The flasks were incubated at static condition for 15 days at 30^{o}C (Vassilev *et al.,* 1993). At the end of incubation time, sugar consumption and BC production were determined.

Reusing of the immobilized *G. xylinus* ATCC 10245 cells

The best immobilized bacterial cells that gave the highest production of BC were reused for several cycles in a fermentation medium containing 10 cubes of LP and supplmented with one ml of bacterial suspensionm incubated at 30^{o}C for 15 days at static conditions.The reused of the adsorbed cells was carried out by removing the medium after 15- days and a new sterilized medium (30 ml) was added, then a 2nd cycle was run at the optimum conditions.this process was repeated. At the end of each cycle, the BC production and sugsr consumption were determined.

Physicochemical properties of the produced BC

The BC powder was mixed with distilled water and allowed to stand with occasional stirring for one hour. Thereafter, the liquid supernatant was collected and the pH of the liquid was tested using pH meter. For solubility test, 250 mg of BC powder were separately mixed with sodium hydroxide, methanol, acetone and cupriethylenediamine (Cuen). The solubility of the fiber in each solvent was then observed (Halib *et al*., 2012). In order to determine the water absorption capacity (WAC), the dried BC samples were immersed in water at room temperature. Then, the samples are taken out from the water and excess water left on the BC surface is blotted out with Kimwipes paper. After that, the weights of the hydrated BC samples were measured. The procedure was repeated till no further weight change (Junaidi *et al*., 2012). The water content is calculated using the following formula: WAC (%) = [(Wh – Wd)/Wd] x 100%, where Wh and Wd indicate the weight of the hydrated and dried BC samples, respectively.

Scanning Electron Microscopy (SEM) analysis

Microscopic photographs of the adsorbed *G. xylinus* on the best solid support and also The BC sample were prepared and then examined using SEM (Jeol.Jsm-5300), (Central Laboratory, Faculty of Science, Alexandria University, Egypt). However, the BC sample was sputtered coated with gold and examined at 15000 × magnification for surface view of BC. The same procedure was carried out for BC dried sheet sample, but using 10000 × magnification (Halib *et al*., 2012).

X-ray analysis

The X-ray diffraction (XRD) was used to examine the crystallinity degree of the BC. It was performed with a Rigaku Smart lab XRD (Rigaku, The woodlands, TX Central Laboratory, Faculty of Science, Alexandria, Egypt) using Cu radiation generated at 40 kv and 44 mA. The Bragg angle of 2θ was scanned from 9° to 41° with a step and exposure time of 5 s. Freeze dried samples 20 mg were used for analysis (Moosavi-Nasab *et al*., 2011). The crystallinity index (C.I.) was calculated from the reflected intensity data using the Segal method, according to the following eqation (Keshk and Sameshima, 2006): C_{Ir} (%) = $\{(I_{002}-I_{am})/I_{002})\}*100$, where I_{002} is the maximum intensity of the lattice diffraction and I_{am} was the intensity at $2\theta = 18^{o}$.

Mechanical properties of the produced BC

The mechanical properties of the BC pellicles were analyzed through uniaxial tensile tests, using a testing machine Zwicki 2.5KN, Sadex Technical Consulting and Agencies, Alexandria, Egypt. Using the Bluehill software, connected to the testing machine, it was sufficient to insert the dimensions of the samples before the beginning of the experiments, and the software calculated automatically the values of stress, strain and Young's modulus during the test (Keshk and Sameshima, 2006).

Applications of the produced BC via formating aerogel

The aerogel was formed to be applied in several potential applications such as; water and oil absorption. Water absorption capability needed to develop the hydrophilic properties of BC aerogel, while the oil absorption nedded to develop the hydrophobic properties of BC aerogel. Furthermore, the adsorption of heavy metal ions from aqueous solutions using BC aerogel was studied.

Development of hydrophilic BC aerogel and water absorption test

The BC aerogel was formed firstly to be used in such test and other followed testes. The BC (2 %) was dispersed into sodium hydroxide/urea solutions (1.9/10, %) by sonicating for 6 min. Thereafter, the solution was placed in a refrigerator for more than 24 h to allow gelation of the solution. After the solution has been frozen, it is then thawed at room temperature and then followed by immersing into ethanol (99%) for coagulation. The specimen thickness was controlled at 1cm with a diameter of 3.5 cm using a beaker as a mold. After coagulation, solvent exchange was carried out by immersing the gel in de-ionized water for two days. The sample was then frozen in a freezer at -18^{o}C for 12 h. After that freeze drying was carried out for two days with freeze dryer to obtain the desired aerogel (Nguyen *et al*., 2014).

Water absorption capability of aerogel samples was investigated in deionoized water. The dry sample dimensions were 40 mm (diameter) × 12 mm (thickness). The dry sample was weighed and immersed in 800 ml of de-ionized water for a certain time. The wet sample was weighed, dried or squeezed and weighed again. The test was repeated

three times with an immersion time of 2 h. To dry the aerogel, it takes seven days for the absorbed water to evaporate naturally under the atmosphere conditions (Nguyen *et al*., 2014).

Development of hydrophobic BC aerogel and oil absorption test

For hydrophobic coating, the BC aerogel was placed in a big glass bottle. A small open glass vial containing methyltrimethoxysilane (MTMS) was added into the glass bottle. Then, the glass bottle was capped and heated in an oven at 70^{o}C for 2 h for the silanation reaction. Thereafter, the coated sample was placed in a vacuum oven to remove the excess coating reagent (Nguyen *et al*., 2014). The test was similar to the water absorption test except that the excess oil was allowed to drain for 30 s after lifting up the wet sample. Motor oil with a viscosity of 130 cP, an olive oil with a viscosity of 100 cP and cooking oil with a viscosity of 55 cP were used in the test (Nguyen *et al*., 2014).

Adsorption of heavy metal ions from aqueous solutions using BC aerogel

Adsorption process was performed under static conditions along 48 h at room temperature using 1 g of adsorbent with 0.1 mm grain-size and 100 ml of metal solution ions (20 mg/l). Concentration of Cd^{2+}, Pb^{2+}, and Cu^{2+} was determined using atomic absorption spectroscopy (Chemical lab, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt). Amount of adsorbed metal was calculated from the equation: $Q = (C_o - C_e)\ V/m$, Where: Q is amount of adsorbate adsorbed on the adsorbent, mg/g; C_o is initial concentration of adsorbate, mg/ml; C_e is concentration of adsorbate at equilibrium state, mg/ml; m is mass of the adsorbent, g; V is volume of aqueous solution, l. **(**Chen *et al*., 2010). Removal efficiency (R) % = $[(C_o - C_e)/C_o] \times 100$.

Biodegradation of the produced BC

To prove the biodegrability of the present BC and then its safety, 100 mg of its sheet were mixed with 50 ml of waster sample from different sources (Nile water and seawater) or mixed with 50 g of marine sediment or garden soil. The efficacy of biodegradation process was detected by determining the total bacterial count (CFU/ml) along 0, 5, 10, and 15 days interval. Also, the glucose concentration was initially estimated as 1 mg/l, and then estimated finally again.

RESULTS

Effect of entrapping cells with different gel materials on BC production

Different gel materials were used for entrapping *G. xylinus* ATCC 10245 cells. The gel entrapping the bacterial cells was usually in the form of beads in the case of Ca-alginate (2%) and k-carragennan (2%), while agar and agarose gel were in the form of small cubes (about 0.5 cm length). The results recorded in Figure 1, supported that the BC production of all entrapped cultures was lower than that of free cell cultures. The BC

produced by entrapped *G. xylinus* ATCC 10245 in alginate beads recorded the highest productivity (12.5 g/l) comparing to other entrapped cells, while the k-carragennan gave the lowest BC production (9.3 g/l).

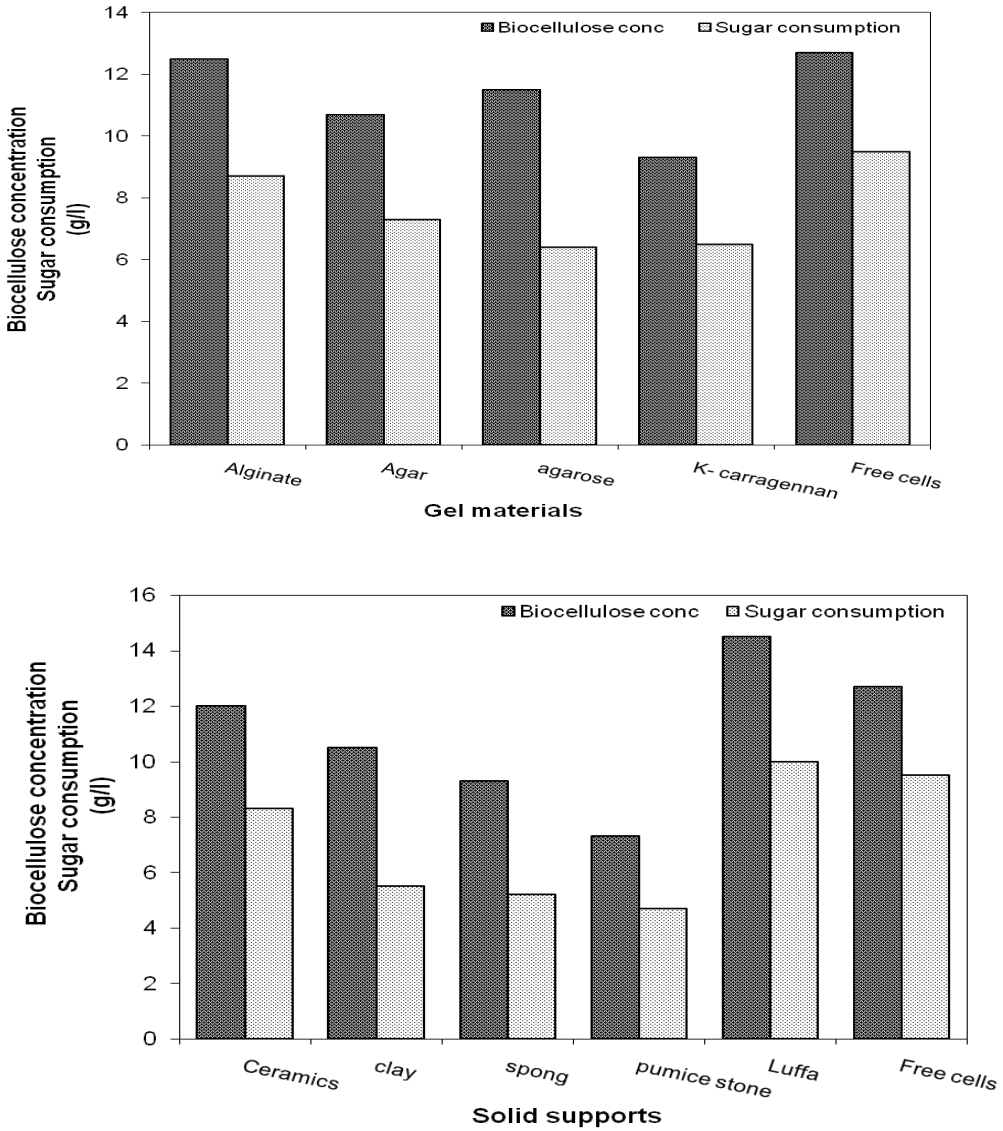

Fig.1: Production of BC and sugar consumption by *G. xylinus* ATCC 10245 entrapped on different gel materials (Upper) and adsorbed on different porous supports (Lower).

Effect of adsorption of *G. xylinus* ATCC 10245 cells on BC production

Data of this experiment conducted to the cells were adsorbed on SSC, LP, PP, CP, and CP. The results graphically illustrated in Figure1,revealed that the adsorption of *G. xylinus* ATCC 10245 cells on LP and CP gave the highest BC production (14.5 g/l) and sugar consumption (10.0 g/l), respectively. This represents increasing of 1.14 and 1.05-fold, respectively from free cells. On the other hand, other solid supporters produced

lower BC production. The illustrated attempt to immobilized *G. xylinus* ATCC 10245 cells by entrapment and adsorption mechanism clarified that LP was the best immobilizing support as it produced the highest BC production, while immobilizing bacterial cells by k-carragennan, pumice, and synthetic sponge recorded the lowest BC production (9.2, 7.3, and 9.5 g/l, respectively).

Production of BC by reused adsorbed *G. xylinus* ATCC 10245 cells

The effect of repeated reuse of LP as the best adsorbed solid support for the BC production by *G. xylinus* ATCC 10245 was examined. The results shown in Figure 2 indicated that the reused of the adsorbed *G. xylinus* ATCC 10245 cells on LP slightly enhanced the BC production in the culture medium of 2nd run (15.7 g/l), then the reused culture showed gradually decrease in the BC production up to the 4th run; where the adsorbed *G. xylinus* ATCC 10245 cells exhibited the lowest BC production (11.5 g/l).

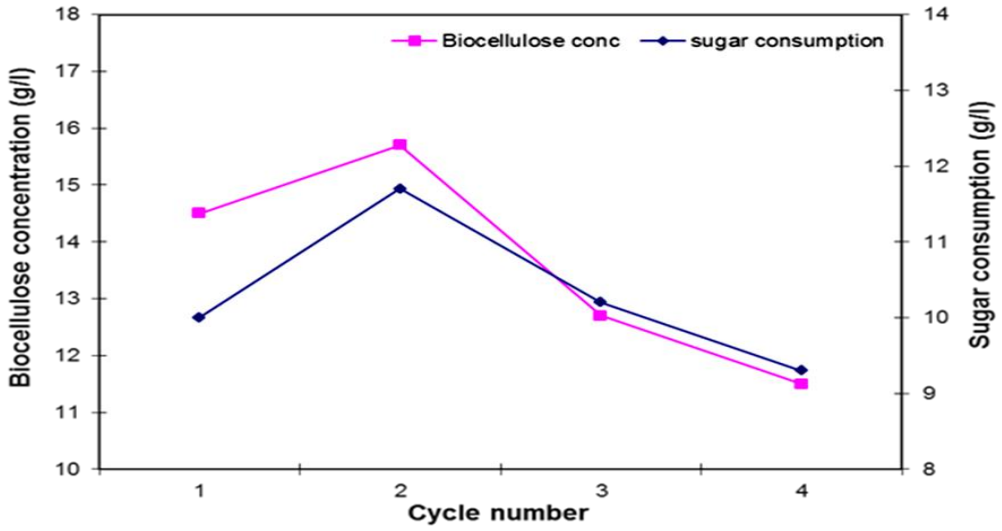

Fig. 2: Production of BC and sugar consumption by reused adsorbed *G. xylinus* ATCC 10245 cells.

SEM micrographs of adsorbed *G. xylinus* ATCC 10245 cells

According to SEM micrographs, Figure 3 shows that there is a high condense of *G. xylinus* cells on surface of the LP. The micrographs indicate the good adsorption of *G. xylinus* cells to the surface of supporting material with different degree, so that, it could emphasis the explanation of high BC production resulted due to adsorption on LP.

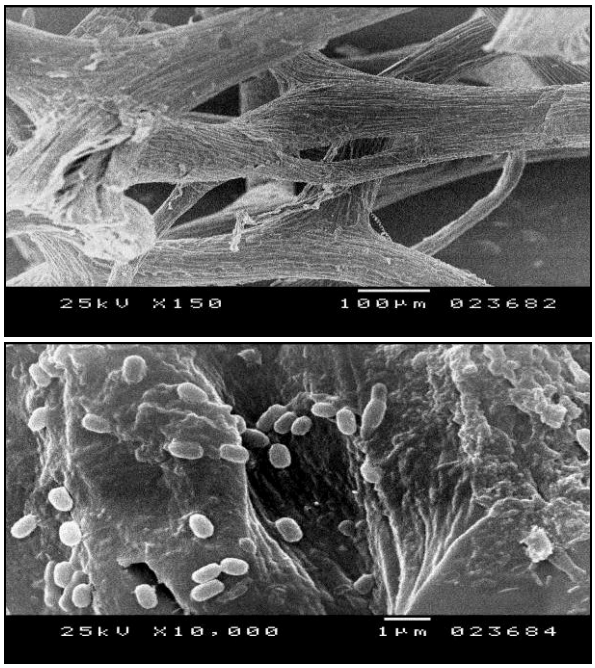

Fig. 3: SEM micrographs showing the structure of LP (Upper), and *G. xylinus* ATCC 10245 cells adsorbed on the surface of LP matrix (Lower)

Physico-chemical properties of *G. xylinus* ATCC 10245 BC

The pH of the supernatant liquid from the BC and distilled water mixture was determined to be pH 7.3. The physical observation showed that the BC powder did not dissolve in solvents such as sodium hydroxide, methanol, and acetone. Indeed, it completely dissolved in cupriethylenediamine (Cuen). The water absorption capacity for each of the BC produced from sea grasses media was 199%.

SEM analysis of G. xylinus ATCC 10245 BC

This analysis was applied to examine the surface features of BC nanofibers produced by *G. xylinus* ATCC 10245. Data represented in Figure 4, exhibited the surface view for the BC nanofiberils, which can't be observed due to converge by thick layers. In paricular, it has well interconnected pore network structure. All these made the BC fibrous with irregular size and shape. It was observed that the BC shown with fine cellulose ribbons, sometimes called fibrils.

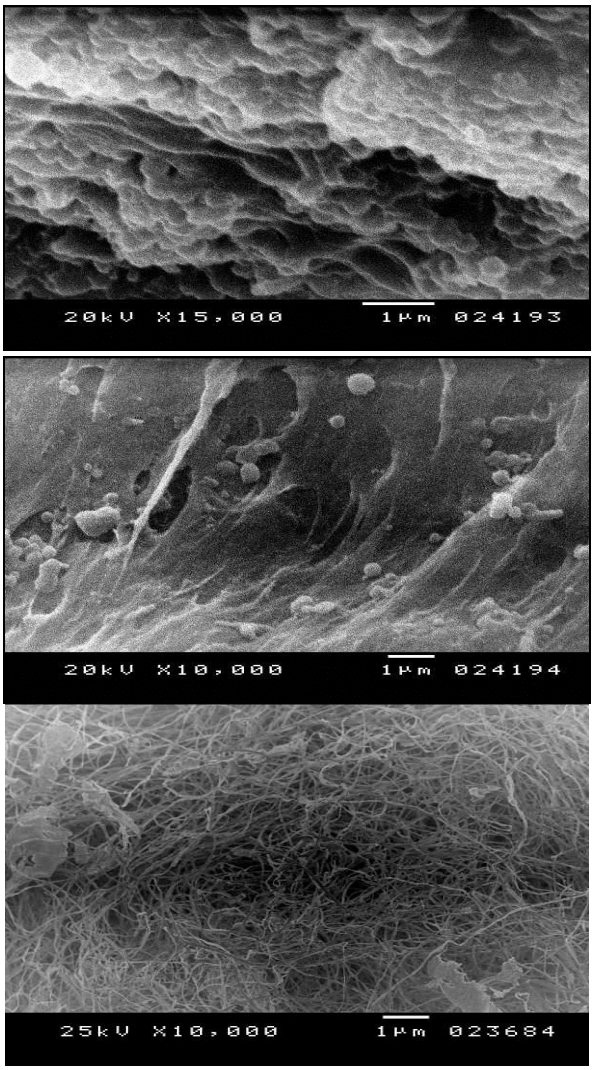

Fig. 4: SEM micrographs of BC produced by *G. xylinus* ATCC 10245; BC surface (Upper), BC dried sheet (Middle) and BC fibril network (Lower)

X-ray analysis of BC produced by G. xylinus ATCC 10245

The crystalline index values of BC were calculated from XRD spectra using amorphous region subtraction. The resulting spectra were analyzed and normalized (Figure 5). The crystallinity for the produced BC was 84%.

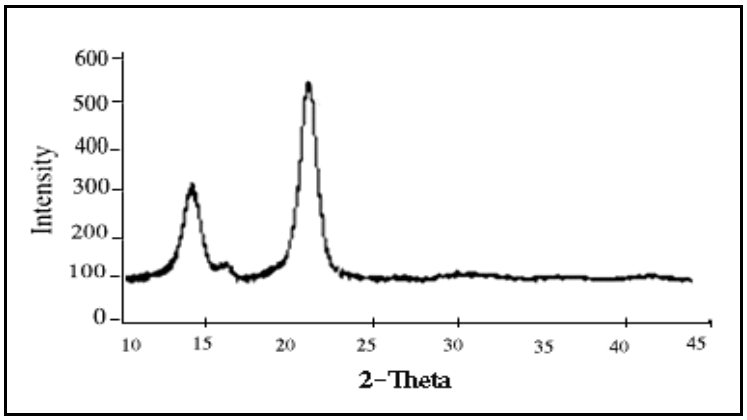

Fig. 5: X-ray patterns of BC produced by *G. xylinus* ATCC 10245.

Tensile strength BC produced by G. xylinus ATCC 10245

Tensile strength of the BC produced by *G. xylinus* ATCC 10245 was determined for studying mechanical properties, which are summarized in Table (1). However, they revealed the good quality of our product.

Table 1: Tensile tests on BC produced by *G. xylinus* ATCC 10245.

Parameter	Value
Specimen thickness (mm)	0.11
Specimen width (mm)	14
Tensile strength (MPa)	22.7
Young's modulus (Mpa)	349.5

Applications of the produced BC by formating aerogels

The formed BC aerogel was used in some significant and valuable applications in rising up the water absorption capability, the oil absorption and adsorpting selected heavy metals from aqueous solutions.

SEM analysis of the produced BC aerogel

It was observed that the BC aerogel and SEM analysis of freeze-dried BC aerogel revealed its highly porous network structure (Figure 6).

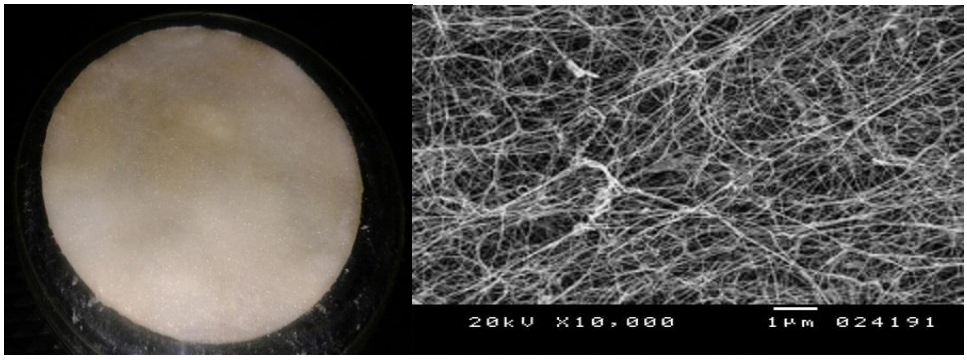

Fig. 6: BC produced by *G. xylinus* ATCC 10245; aerogel (Left) and SEM image of BC aerogel (Right).

Water absorption test

The water absorption of BC aerogel was studied in two phases. In the first phase, water absorption ability of BC aerogel was measured each 15 min for 2 h. In the second one, the aerogel sample was subjected to three cycles of water absorption tests using a dip coater for 2 h. The weight of the sample was measured before and after each test (Table 2).

Table 2: Water absorption test with various absorbance times.

Phase	**m_d (g)**	**m_w (g)**	**m_s (g)**	**WAC (%)**
First absorption	1	7.3	0.97	630
Second absorption	0.97	7	0.97	622
Third absorption	0.95	6.8	0.95	616

m_d, weight of initial sample; m_w, weight of wet sample; m_s, weight of dried or squeezed sample.

Oil absorption test

The MTMS-coated BC aerogel exhibited strong affinities to the motor, olive, and cooking oils with high oil uptake contents of 7.5, 5.2, and 3.7 gg^{-1}, respectively (Fig.7).

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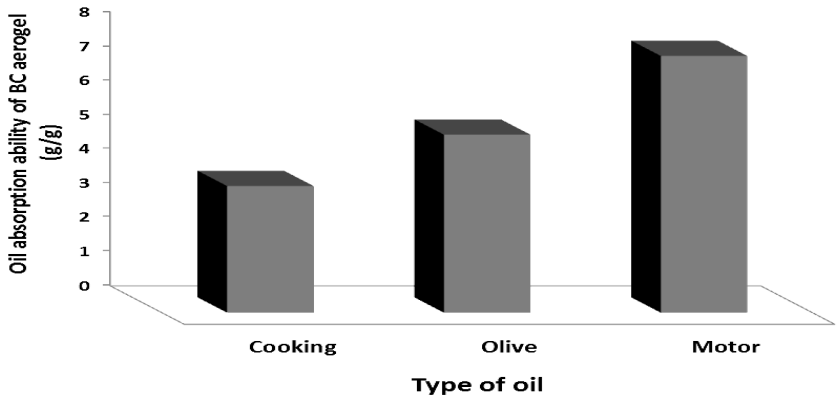

Fig.7: Oil absorption ability of the BC aerogel towards motor, olive, and cooking oils.

Adsorption of heavy metal ions from aqueous solutions using BC aerogel

The BC aerogel was characterized by high sorption capacity for Cd^{2+} comparing to other metal ions. It showed the highest capability of Cd^{2+} removal (95.7%) at pH 6 (Table 3).

Table 3: Removal efficiency and sorption capacity of heavy metals on BC aerogel at different pHs.

pH	Removal efficiency (R %)			Sorption capacity; Q (mg/g)		
	Pb^{2+}	Cd^{2+}	Cu^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}
4	70	94.7	94.1	1.41	1.89	1.88
5	74	95.5	94	1.48	1.9	1.88
6	76	95.7	94.6	1.52	1.91	1.89
7	68	94.4	93.7	1.36	1.88	1.87

Biodegradation of the produced BC

The biological effect of some means such as freshwater, seawater, marine sediment, and garden soil microbes was determined on the BC produced by *G. xylinus* ATCC 10245. The total bacterial count increased periodically between zero and 15-days incubation. The BC with the Nile water had the highest count ($1.2x10^{12}$ CFU/ml), followed by the garden soil treatment ($1.1x10^{11}$ CFU/ml), while, the other two treatments had count around $1.0x10^{9}$ CFU/ml (data not shown). The final glucose concentration after 15-days incubation was 17, 15, 10, and 7 mg/l, for Nile water, seawater, soil, and marine sediment, respectively. However, it was shown that freshwater microbes had the highest effect for the BC biodegradation (Figure 8).

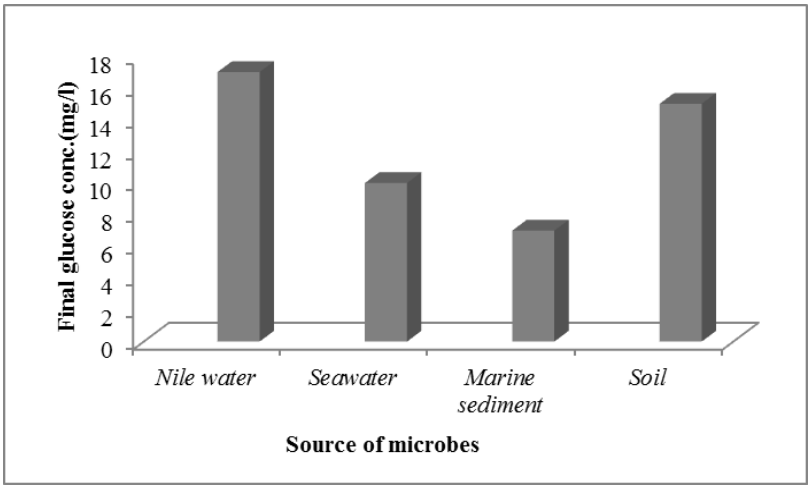

Fig. 8: The final glucose concentration as a factor for the biodegradation of the BC produced by *G. xylinus* using different environmental microbes.

DISCUSSION

Gluconacetobacter xylinus ATCC 10245 is usually the most employed microorganism for producing biocellulose because of its ability to produce relatively high levels of polymer from a wide range of carbon and nitrogen sources (Bielecki *et al*., 2005). This bacterium produces cellulose from a wide variety of substrates and is devoid of cellulase activity (Chawla *et al.,* 2009; Hungund *et al.,* 2013).

Several workers (Zeng *et al*., 2011; Wu and Liu, 2012) have used the batch culture for the BC production. Batch culture is preferred having fewer parameters to be controlled and well understood. So, all experiments during this study were done in batch culture.

The cheaper substrate used instead of the commonly used sugars as glucose or fructose might result in a lower cost of the final product. So, the medium with reducing sugars came from saccharification of *U. lactuca* supported the highest production of BC (3.7 g/l) (Beliah, 2017).

The immobilized *G. xylinus* cells by different techniques (entrapment and adsorption) were applied for BC synthesis. The production of biopolymers by immobilized bacteria using different materials were studied well (Berekaa *et al*., 2009). Reusing of microbial cells aimed to enhance the cell population and activity (Chitturi and Lakshmi, 2016).

The works of both Suwannapinun *et al*. (2007) and Enas *et al*. (2015) had reported that the native *Gluconacetobacter* pellicle had mechanical properties including shape retention and tear resistance that was superior to many synthetic fibers. According to British Pharmacopoeia, pure cellulose should have pH of supernatant liquid around pH (5-7.5) (Halib *et al*., 2012).

The powder completely dissolved in cupriethylenediamine (Cuen), was considered the best well known solvent for cellulose (Halib *et al*., 2012). As well as, ethylenediamine is a good swelling agent for cellulose. By disrupting intermolecular hydrogen bond, swelling promotes molecular chain separation allowing thorough penetration of the solvent. This process had enhanced the complex formation with cellulose glycol groups. The complex is sufficiently stable to prevent aggregation of chains and formation of precipitation. Therefore cellulose was being dissolved (Johnson, 1985). Hence the results supported that cellulose is the primary component of the bacterial cellulose powder.

The WHC is considered one of the most important physical characteristics of bacterial cellulose pellicles regarding biomedical application of BC as wound dressing material. Biocellulose has a high WHC in compared to cellulose plant. This is due to the fact that hydrogen bonding is stronger and more BC cellulose chain (n) long from plant cellulose (Mohammad *et al*., 2014). The variations between the WHC are related to the porosity and surface area of each BC. The greater the surface area and the pore size the larger the amount of water penetrated and trapped in the BC matrix (Ul-Islam *et al*.,

2012). The WHC of our BC provides a special nano-morphology results that lead to the remarkable water absorbing ability of the BC. These findings are in agreement with previous reports showing that microbial cells have the potential to absorb more than 100 times their dry weight in water (Lin *et al*., 2009). The WHC of the BC produced in the current study are in good agreement with study of Ullah *et al*. (2016) and sometimes even higher (Jung *et al*., 2005).

The SEM analysis of indicates that the BC surface nanofiberils can not be observed due to converge by a thick layers. So, it has well interconnected pore network structure (and BC dried sheet, the biocellulose was fibrous with irregular size and shape. Similar observation has been previously reported by Nge *et al*. (2010) and Halib *et al*. (2012).

Obviously, the BC fibril network produced by *G. xylinus* ATCC 10245 was observed. The fibers were loosely arranged with large pores in BC. Moreover, a randomly distributed condensed network of fibrils was observed. A magnified image (1 μ) further clarified that the fibrils were thin and possessed plenty of gaps between them. Similar observation has been previously reported by Ullah *et al*. (2016). SEM images of BC fibers, showed more delicacy. The higher delicacy of the BC fibers could influence its properties such as WHC, thermal stability, and mechanical strength. For instance BC has a higher tensile strength, mold ability and WHC (Jung *et al*., 2005).

Biocellulose is a semi-crystalline material that usually produces three major characteristic crystallinic peaks when examined through XRD (Ullah *et al*., 2016). It showed that XRD spectrum for our BC was similar to advanced reports (French, 2014; Khan *et al*., 2015). Moreover, the crystallinity degree of the produced BC in this study was in good agreement with work of Tsouko *et al*. (2015) and sometimes even higher (Ullah *et al*., 2016).

The tensile strength and young's modulus of our BC were similar to obtained by Suwanposri *et al*. (2014) and sometimes even higher than those obtained by Ullah *et al*. (2016). However, the fibers of BC are thicker, and more compact. The thicker fibers and compact structure could be attributed to the continuous production and aggregation of glucose chains with the passage of time as reported previously by Mormino and Bungay (2003). This compactness and uniform arrangement of fibrils in BC could favor a uniform response to the applied force and result in its improved tensile strength (Ul-Islam *et al*., 2012). The nano sized network wires promotes high surface area, Due to its properties BC can be used in nature, like nanocrystals form or associated with other natural or synthetic polymers to obtain biodegradable materials (Schröpfer *et al*., 2015).

Since bacterial cellulose has many properties like high degree of crystallinity, high purity, good shape retention, high density, high water binding capacity, and higher surface area as compared to the native cellulose, so it can be used in various areas (Iguchi *et al*., 2000). In particular, the formed BC aerogels were dried gels with high porosities, large surface areas, and extremely light weights. It is an ideal material for formation

aerogel due to its renewable, biocompatible, and biodegradable properties. The aerogel was prepared using a sodium hydroxide/urea method (Isobe *et al*., 2012) due to the low cost and convenience of the technique.

When the BC is coated with MTMS, it will become hydrophobic and oleophilic and therefore, has a good affinity to oil. The MTMS-coated BC aerogel was used for the oil absorption test. Water and oil absorption tests showed that the uncoated and the coated aerogels have high liquid absorption capacities of 6-7 times their own weights and can be easily reused by a simple squeezing or drying to remove the absorbed liquid (Nguyen *et al*., 2014).

There is a demand to synthesize new absorbent materials with high absorption capacity and easy water removal (Zhang *et al*., 2012). Additionally, the aerogels are dried gels with high porosities, large surface areas, and extremely light weights (Baetens *et al*., 2011). Consequently, the BC produced by *G. xylinus* applied in aerogel formation. Water absorbed by the BC aerogel is removed easily by simply squeezing it (Nguyen *et al*., 2014). Although no cross-linkers were used in the synthesis, the aerogel preserved its shape after being immersed in water for 2 h, indicating that the material has a stable structure due to the cellulose-cellulose hydrogen bonding (Chen *et al*., 2011). Furthermore, the BC based aerogels have also been used as adsorbents to remove pollutants from aqueous systems. Zhao *et al*. (2015) cellulose aerogels with high porosity and large specific surface area have been used for Cr (VI) removal.

On the other side, the pH of the solution had been reported to be important factor in adsorption processes. The variations in adsorption capacity of BC aerogel with increasing pH could be inferred that the adsorption capacity increased as solution pH increased from pH 4 to 6 and capacity decreases above pH 6. Similar observation has been previously reported by Luzny *et al*. (2014). With an increase in the pH, the negative charge density on the adsorbent increased due to the deprotonation of the metal binding sites, leading to the increase of metal adsorption (Salehzadeh, 2013).

Finally, this work was extended to investigate the fate of the *G. xylinus* BC in the environment. Our data exhibited that fresh water microbes had highest effect for BC biodegradation within 15 days. Basically, the bacteria, fungi, and actinomycetes can degrade cellulosic wastes in the environment to use them as a source of carbon and energy include a variety of aerobes and anaerobes, mesophiles and thermopiles (Gold *et al*., 2000; Gutierrez *et al*., 2001). It has been reported that several fungi such as *Trichoderm spp, Aspergillus spp, Aspergillus chaetomium, Aspergillus fumigatus, Aspergillus flavus, Curvularia, Fusarium, Memoniella* sp, *Phomo* sp, *Thielavia* sp., and *Penicillium* spp. degraded cellulose (Makeshkumar and Mahalingam, 2011; Wilson, 2011). Also, results obtained by Srikandace *et al.* (2018) showed that fungi biodegraded BC films through broth fermentation whereas no biodegradation activity on solid substrate fermentation. Collins and Charles (2017) identified cellulose-degrading bacteria as *Bacillus brevis, Bacillus coagulans, Bacillus laterosporus, Bacillus firmus,*

Pseudomonas mallei, *Pseudomonas putida*, *Cellulomonas fimi*, and *Cellulomonoas flavigena* from the soil containing old rice husk dump within 60 days. All those support the safe usage of the BC produced by *G. xylinus* ATCC 10245 on the environment.

CONCLUSION

Aiming to improve BC production, the cell immobilization of *G. xylinus* by entrapment or adsorption techniques was studied. The highest BC productivity (14.5 g/l) was observed in cells immobilized LP as a solid support. The productivity was about 1.14-fold of that obtained from free cells. The immobilized cells were reused for 2 successive cycles covering a period of 30 days. At second cycle on LP, the BC production reached about 15.7 g/l, and about 1.24-fold. However, a gradual decrease in BC production was observed in the next reuses. Scanning electron microscope indicated the good adsorption of bacterial cells to the surface of LP with different degrees.

Some properties of the BC produced by *G. xylinus* were studied well. Significantly, it had high water absorption capacity (199%) with good tensile strength (22.7 MPa) and young's modulus (349.5 MPa). So, these properties enable *G. xylinus* BC for a potential application in paper industry to produce parchment paper.

On the other side, the formation of BC aerogels was conducted and its characteistics of water absorption capacity and oil absorption ability qualify to be used in diapers industry. In addition, the adsorption potential of BC aerogel in the removal of heavy metal ions (Pb^{2+}, Cd^{2+}, and Cu^{2+}) from aqueous solution.

Inclusively, this work may provide relevant scientific information about the usage of produced BC by *G. xylinus* cells in the Egyptian market, such as a paper-making industry, and diapers. Because the aerogels have ability to absorb oil spills, they can be effectively used in the purification and treatment of wastewater from heavy metals.

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