Production of bacterial cellulose by *Gluconacetobacter hansenii* UAC09 using coffee cherry husk

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Revised: 20 April 2011 / Accepted: 26 April 2011 / Published online: 28 May 2011

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Abstract The work is aimed to investigate the suitability of underutilized coffee cherry husk (CCH) for the production and optimization of bacterial cellulose (BC) by *Gluconacetobacter hansenii* UAC09 and to study the physico-mechanical properties of BC films. CCH extract was used as a carbon source in various concentrations along with other nutritional components such as nitrogen (corn steep liquor, urea) and additives (ethyl alcohol, acetic acid). Concentration of CCH extract at 1:1 (w/v) along with 8% (v/v) corn steep liquor, 0.2% (w/v) urea, combination of 1.5% ethyl alcohol and 1.0% (v/v) acetic acid resulted in the production of 5.6–8.2 g/L of BC. BC had tensile strength varying between 28.5 and 42.4 MPa. BC produced with CCH and Hestrin and Schramm (HS) media did not differ in structure as analyzed by FT-IR. Scanning electron microscopic studies indicated BC to contain reticulated network of fine fibers. Under optimized condition, based on the other additives, CCH produced more than three folds yield of BC (5.6–8.2 g/L) than control medium (1.5 g/L). This is the first report on the use of CCH for the production of BC and paved way for the utilization of organic wastes with pectin and high polyphenol content.

Keywords Coffee cherry husk · Bacterial cellulose · *Gluconacetobacter* · Ethanol · Acetic acid

Electronic supplementary material The online version of this article (doi:10.1007/s13197-011-0401-5) contains supplementary material, which is available to authorized users.

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Introduction

Production of bacterial cellulose (BC) is a characteristic feature of *Gluconacetobacter* sp. It is produced as a pure polymer without any other components as found in plant cellulose, which is usually associated with hemicellulose and lignin. Highly crystalline nature and strong hydrogen bonds of cellulose make it insoluble in most of the solvents. The chemicals used in the solubilization of cellulose from plant biomass are corrosive and detrimental causing environmental pollution. In this regard, cellulose by *Gluconacetobacter* sp. has created a great interest in the scientific community. The BC is more convenient to be produced and can be processed into pure form with simple alkali treatment (George et al. 2005b).

BC displays many unique properties including high mechanical strength, crystallinity with stability towards chemicals and high temperature. In native state, it has good hydration, holding water over 100 times its own weight. The improved BC properties are due to the reticulated network of fine fibers, with a diameter of 0.1 μm , which is about one hundredth that of plant derived fibers. All these properties make the BC far superior to its counterpart from plant origins. BC is used in many special applications such as, scaffold for tissue engineering of cartilages (Svensson et al. 2005), blood vessels, as an artificial skin during burns and wounds (Krystynowicz et al. 2002). It is considered as one of the traditional foods known as ‘natto’ among Philippine people and is popular in other Asian countries, including Indonesia, Japan and Taiwan, due to its distinctly soft texture and high fiber content (Ochaikul et al. 2006).

Cost of production of BC is high due to the high input cost in terms of raw materials and low yields (Moon et al. 2006). There are many reports on efficient utilization of

agro-industrial residues for the production of value added products (Kumar et al. 2010; Sharma et al. 2010).

CCH is one of the most abundantly available agro-industrial wastes produced after processing of coffee cherry by dry process. For every ton of coffee cherries, nearly 0.18 ton of CCH is produced (Adams and Dougans 1981). CCH contains carbohydrates, proteins, minerals and high amount of polyphenols. Due to the presence of unfavorable substances like caffeine, tannins and other polyphenols, its use in agriculture has been restricted to a large extent and the disposal of coffee waste causes an enormous pollution problem in the processing units (Venugopal et al. 2004). Application of CCH in bioprocess at one hand provides cheaper alternative substrate and on the other hand it helps to solve pollution problems.

The present work was undertaken for the utilization of CCH and corn steep liquor (industrial by-product), as an alternative to the conventional synthetic media, for enhanced BC production and to study the physico-mechanical properties of the BC films produced under optimized conditions.

Materials and methods

Microorganism The organism used in this study was isolated from contaminated grape wine. The pellicle formed in contaminated grape wine was collected and washed with sterile distilled water and cut into pieces (1 cm²) under aseptic conditions. Cut pieces were placed in a sterile plastic bag containing 0.1% peptone saline solution. To obtain a cell suspension, the contents were pressed manually to release the entrapped cells (Verschuren et al. 2000), serially diluted and streaked on Hestrin and Schramm (HS) agar plates (Schramm and Hestrin 1954) and the plates were incubated at ambient temperature in inverted position for 48–72 h. Isolated colonies were picked and further purified. The purified culture was inoculated into sterilized grape extract and reconfirmed for its ability to produce pellicle. The culture was subjected to morphological and biochemical tests as per the standard microbiological methods (Sievers and Swings 1984). The organism was maintained on HS medium slants at 4 °C and sub cultured at intervals of 2 weeks.

Molecular characterization Total genomic DNA was extracted from overnight grown culture broth using standard method (Sambrook et al. 1989). PCR was carried out to amplify the targeted 16S rRNA gene. The template DNA was amplified using 5' GAGTTTGATCCTGGCTCAGG 3' and 5' TCATCTGTCCACCTTCGGC 3' primers obtained from Sigma Aldrich, India. A total volume of 25 µl reaction mixture containing 10 ng template DNA, 5 µl dNTP's of 10 mM, 2.5 µl of 10x taq buffer, 2 µl primers of 10 µM concentration, 1 µl taq DNA polymerase. The mixture was

made up to a final volume of 25 µl. PCR amplification was performed in an automated DNA thermal cycler (Primus 96, UK). Reaction was carried out by using an initial denaturation of 94 °C for 4 min followed by 34 cycles of denaturation at 94 °C for 40 s; annealing temperature of 56 °C for 40 s; and elongation at 72 °C for 1 min 30 s; final extension by 72 °C for 1 min. The PCR product was run in 1.2% agarose gel for 1.5 h at 100 V, stained in 0.5 µg/ml ethidium bromide solution and documented in gel documentation system (Bio Rad, Universal Hood, Italy). The amplified PCR product was purified using commercially available PCR purification spin kit as per the manufacturer's instructions (Hipur A, Himedia Laboratories, India) and subjected to sequence analysis (Bangalore Genei, India). The resultant nucleotide sequence was subjected to BLAST programme of NCBI (online access) to assess the percent homology with closely related strains documented in gene bank data bases. The data generated was further processed by multi aligning of the sequences of different species of *Gluconacetobacter* (Clustal W multiple alignment in bioedit sequence alignment editor) and a phylogenetic tree (Fig. 1) was constructed (Mega 3.1). The sequence was deposited in gene bank (National Center for Biotechnology Information Taxonomy, Bethesda, USA) with an accession no. *G. hansenii* UAC09 (FJ655878).

Production media HS medium (g/L) contained glucose-20, peptone-5, yeast extract-5, citric acid-1.15, di-sodium hydrogen phosphate-2.7, pH-4.5 (Schramm and Hestrin 1954).

CCH of coffee variety robusta was collected locally from Kodagu district, Karnataka, India. Cleaned CCH was dried to 12% moisture and ground to 50 mesh size in a plate mill. Powdered CCH was boiled with 1:1(w/v) distilled water for 30 min. The thick viscous slurry was filtered through muslin cloth and used in all the experiments. Physical and chemical parameters such as pH, total sugar (Sawhney 2006) and total polyphenols (Yuan et al. 2005) of CCH extract were determined.

Inoculum preparation One loopful of culture from the stock was inoculated into sterile HS broth and grown on a rotary shaker (150 rpm) at 27±1 °C for 24 h. The resulting suspension was used as an inoculum (5% v/v) for all the experiments and inoculated flasks (100 ml medium in 500 ml flask) were incubated at 27±1 °C in stationary condition for 2 weeks. All the experiments were conducted in triplicate.

Media optimization To study the effect of CCH concentration on BC production, various dilutions (1:1–1:6 w/v) of CCH extract were prepared with distilled water individually. The effect of various nitrogen sources on BC production like urea (0.1–0.5% w/v) and corn steep liquor (2–10% v/v) were tested

Fig. 1 Phylogenetic tree constructed for 16S rRNA gene of representative *Gluconacetobacter* species

individually along with CCH extract. The total solid content of corn steep liquor (CSL) was estimated (El-Saied et al. 2008). At the time of inoculation, ethanol (1.0, 1.5 and 2.0% v/v) and acetic acid (0.25, 0.5, 1.0 and 1.5% v/v) were added to the sterilized media and incubated as explained above.

Purification of pellicle Pellicle grown on the liquid surface was harvested once a week under sterile condition. The pellicle was washed thoroughly with water, immersed in 1 N NaOH for one day at room temperature to remove the cells and other impurities embedded in the pellicle and rinsed thoroughly with water until a neutral pH was attained in the drained water. The pellicle was press dried in between the filter papers at 60 °C till the film weight became constant (Yoshino et al. 1996; Ramana et al. 2000). Analysis was carried out using the dried BC films produced under various combinations of optimized conditions. The physico-chemical properties of BC obtained under varied fermentation conditions were compared with BC produced from CCH and HS media. All physico-chemical analyses were carried out for both the films (first week and second week harvest) and expressed as an average of both the results.

Scanning electron microscopy Both native and NaOH washed wet pellicles were treated with glutaraldehyde (2.5% v/v) and freeze-dried. Both the samples were gold coated and examined for their surface morphology using

scanning electron microscope (LEO 435 VP Electron Microscope Ltd., Cambridge, UK).

Film thickness The thickness of the press dried films was measured by using Micrometer (Model 549E, Testing Machines Inc. USA). The results were expressed in micrometer, as 100 gauge is equal to 25 μ . BC strips of size 1.5 $\times$ 10 cm were cut and thickness was measured in 5 randomly chosen areas in each strip, the same strips were further used for tensile testing (tensile specimens) and the mean value was taken for tensile strength (TS) calculation.

FT-IR Spectral studies Thin film with uniform thickness was used for obtaining the IR spectra of BC film using IR spectrophotometer (FTIR-RAMAN Nicolet 5700, USA). The measurement was carried out at 20 °C in anhydrous condition with air as the background. For each sample, 32 scans at 2 cm^{-1} resolution were collected in the scanning range of 4000–400 cm^{-1} wavelength.

Mechanical properties To determine tensile strength and percent elongation at break, ASTM D 882/1995 method was followed using LLOYD's universal testing instrument (LLOYDS-50 K, London, U.K.). Specimens of BC films of size 1.5 $\times$ 10 cm were used. Initial grip separation was set at 50 mm with cross head speed of 100 mm/min. TS was calculated by dividing the maximum load for breaking the

film by cross-sectional area and % Elongation (%E) by dividing film elongation at rupture to initial gauge length at an ambient temperature (25 ± 2 °C). An average of 10 measurements is reported. Finally, results of TS and %E were calculated for 25 μ thickness of BC films for comparison.

Statistical analysis The experimental data obtained for different parameters ($n=3$) were subjected to analysis of variance (anova). In case of significant difference, mean separation was accomplished by Tukey's highest significant difference (HSD) test using statistica (Statsoft 1999).

Results and discussion

Identification of species Based on the morphological and biochemical characters, the isolate was identified as *Gluconacetobacter* species as per Bergey's manual. The major differentiating biochemical characters were; gram –ve rod, catalase +ve, oxidase –ve, growth on acetic acid and ethanol (3.5%) +ve and production of cellulose +ve. Further analysis of 16S rRNA gene was carried out to confirm the identity. Approximately 1.4 kb fragment of the 16S rRNA gene was amplified and sequenced. On the basis of sequence analysis, it showed 100% homology with *G. hansenii* appearing in the same branch of generated tree (Fig. 1). The closest species of *G. hansenii* was *G. kombuchae* as per the phylogenetic tree. The organism was deposited at National collection of industrial microorganism, NCL, Pune (NCIM) with an accession no NCIM 5415.

Use of CCH as a carbon source Coffee cherry husk is one of the most abundantly available agro- industrial wastes produced after processing of coffee cherry by the dry process in many coffee producing areas (Zuluaga-Vasco 1989). Due to its low utility, Coffee-curing works dump coffee cherry husk in heaps and allow them to rot. The coffee cherry husk damps during and after the rainy season leach coffee cherry husk effluent as a thick viscous liquid. The area in which coffee cherry husk effluent flows was found to be devoid of any vegetation. On initial survey it was recorded that the liquor when diluted (1:10 w/w) kills all the plants including grasses within a period of 10 days due to the presence of polyphenols (Venugopal et al. 2004). There are no reports of coffee cherry husk being used for the production of BC; though, there are many reports describing the utilization of coffee cherry husk and coffee pulp to produce enzymes (Boccas et al. 1994)

Production of BC is a characteristic feature of *Gluconacetobacter* sp. Initial total sugar of the CCH extract at 1:1 dilution was 7.2%, with the total polyphenol content at 0.8% and 4.6 pH. Further dilutions reduced the sugar concentration resulting in lower yield.

Initially, when the suitability of CCH extract was checked for the production of BC, CCH extract with 1:5 (w/v) dilution resulted in 1.6 g/L of BC, which was comparable to 1.7 g/L of BC produced from HS medium by *Gluconacetobacter hansenii* UAC09 (Usha Rani et al. 2011). Dilution of CCH extract had significant effect on BC production. Though BC production was noticed at 1:5 and 1:6 (w/v) dilutions (Fig. 2a), the maximum production of

Fig. 2 Effect of concentration of coffee cherry husk extract (a) addition of corn steep liquor (b) urea (c) ethanol and acetic acid (d) on production of bacterial cellulose by *Gluconacetobacter hansenii* UAC 09 ($n=3$)
□ Bacterial cellulose; -pH

5.6 g/L was observed with 1:1 dilution, which is a threefold increase as compared to HS medium. Hence, all further experiments were carried out with 1:1 dilution of CCH.

This present work has clearly brought out the use of CCH as a carbon source for the production of BC, which was not reported earlier as per the knowledge of the authors. It is evident from the present results that CCH extract, which has up to 7% of total sugar content, is a good source of carbon to support the growth and production of BC by *G. hansenii* UAC09. Earlier work indicated the optimum sugar concentration for BC production to be in the range of 2–4% (Hutchens et al. 2007). More than 4% of total sugar resulted in decreased production of BC, due to the accumulation of gluconic acid, which lowers the pH of the medium and inhibits cellulose production (Son et al. 2001). In contrast, *G. hansenii* UAC09 produced enhanced BC from high sugar (7.2%) containing CCH medium. Mikkelsen et al. (2009) have observed that these bacteria have the ability to produce glucose from various carbon substrates followed by glucose polymerization to cellulose. As majority of sugars in CCH are pectins, utilization of pectin for the production of BC needs to be further investigated. Few nitrogen fixers of the genera *Gluconacetobacter* are known to utilize pectins (Adriano-Anaya et al. 2005), but there are no reports of pectin utilization by *G. hansenii*. The polyphenols act as stimulators of cellulose production by preventing c-di-GMP (the most important factor in cellulose synthesis) from being destroyed by the enzyme phosphodiesterases (Nguyen et al. 2008). However, earlier study had indicated that the total polyphenols above 0.6% retards the growth of *Gluconacetobacter* sp. (Nguyen et al. 2008). But in the present study, BC production was observed at 0.8% of polyphenols, indicating the ability of the isolate to mitigate the toxic effect of polyphenols. This formed the main basis of selecting this isolate for the production of BC using CCH. Earlier workers had used molasses, containing the polyphenolic compounds, as a cheap source of carbon (Keshk and Sameshima 2006). CCH is much cheaper than molasses and it is available throughout the year. The antioxidant properties of phenolics present in molasses, enhance BC production by decreasing gluconic acid concentration (Keshk and Sameshima 2006). Similar result was observed with CCH. The pH of the CCH containing media increased from 4.6 to 5.5 during fermentation irrespective of other nutrients. This is in contrast with the HS medium, where the pH decreased to 4.2

Effect of nitrogen source In an earlier study, it was concluded that CSL is the nitrogen of choice (Usha Rani and Anu Appaiah 2011). Of the two nitrogen sources tested i.e., CSL and urea, CSL was found to be suitable for BC production by *G. hansenii* UAC09 (Fig. 2). The CSL, a cheaper industrial by-product, is a complex organic

nitrogen source, rich in protein, sugars, vitamins, inorganic ions and myo-inositol phosphates (El-Saied et al. 2008). The CSL used in this study had 33.7% total solid content. Addition of CSL (2–10%) to CCH extract resulted in enhanced BC production. Among various concentrations used, combination of CCH and 8% CSL was found to be optimum with a 47% increase in production of BC compared to (1:1) CCH. The increase in BC production may be due the presence of factors that promote cellulose synthesis in CSL. Earlier, Matsuoka et al. (1996) observed that the presence of lactate and methionine in CSL are effective components for enhanced cellulose production and they have reported 4.0 g/L BC by using CSL in basal medium from *Acetobacter xylinum* subsp. *sacrofermentans* BPR2001. Earlier it was reported that enhanced BC production is due to the energy released by the oxidative reaction of lactate to pyruvate (Naritomi et al. 1998). El-Saied et al. (2008) have reported 4.4 g/L of BC by *G. xylinus* (ATCC 10245) from CSL medium using glucose and 4.7 g/L of BC with molasses. The BC produced (8.2 g/L) in the present study is more than the earlier results reported under stationary condition. When urea (0.2%) was used as a source of nitrogen, a 17% enhancement of BC production was observed. Further increase in the CSL and urea concentration had no effect on BC production (Fig. 2b and c).

Effect of ethanol and acetic acid The supplementation of ethanol and acetic acid to the CCH extract increased the rate of BC production. Among various combinations used 1.5% ethanol and 1.0% acetic acid exhibited 24% increase in the yield (Fig. 2d). Earlier, 1.7 times increased production of BC (2.5 g/L) by *G. hansenii* PJK by the addition of 1% ethanol to the basal medium was reported (Park et al. 2003). Increased production was due to the effect of ethanol, which prevents the bacteria from forming a non cellulose mutant (Hutchens et al. 2007) and also it stimulates cell growth and functions as an energy source for ATP generation. The increased levels of ATP leading to abundant flow of G6P (a precursor of BC) into the BC biosynthetic pathway by inhibition of G6PDs led to high yield (Naritomi et al. 1998). Cellulose synthesis competes for glucose with glucose oxidation. Membrane bound glucose dehydrogenase bring in the glucose oxidation reaction which in turn supplies electrons for metabolic processes. Repression of glucose oxidation by addition of ethanol and acetic acid as an alternative electron source has been reported to increase cellulose yield (Velasco-Bedran and Lopez-Isunza 2007).

The observations strongly explain the improved BC production by ethanol, acetic acid and CSL supplementation when used individually. However, in a combination of all three, BC production was reduced (7.5 g/L) and addition

Table 1 Physico-mechanical properties of bacterial cellulose films produced by *Gluconacetobacter hansenii* UAC09 under optimized conditions

Media Composition	BC (g/L)	Final pH	Tensile strength (MPa/25 μ)	Elongation (mm/25 μ)	Thickness (μ)
CCH+8% CSL	8.2 $\pm$ 0.39 ^a	5.4 $\pm$ 0.23 ^a	37.8 $\pm$ 1.22 ^a	0.45 $\pm$ 0.17 ^a	54.3 $\pm$ 3.93 ^a
CCH+0.2% urea	6.5 $\pm$ 0.06 ^b	5.5 $\pm$ 0.08 ^a	42.2 $\pm$ 2.74 ^b	0.38 $\pm$ 0.10 ^a	46.2 $\pm$ 3.93 ^a
CCH+EA+AA	6.9 $\pm$ 0.03 ^c	5.2 $\pm$ 0.06 ^b	39.9 $\pm$ 1.54 ^a	0.54 $\pm$ 0.17 ^a	36.2 $\pm$ 2.05 ^b
CCH+8% CSL+EA+AA	7.5 $\pm$ 0.07 ^d	5.1 $\pm$ 0.04 ^b	42.4 $\pm$ 2.55 ^b	0.58 $\pm$ 0.08 ^b	47.5 $\pm$ 2.09 ^a
CCH+0.2% urea+EA+AA	6.6 $\pm$ 0.07 ^b	5.5 $\pm$ 0.03 ^a	37.9 $\pm$ 2.67 ^a	0.58 $\pm$ 0.11 ^a	44.6 $\pm$ 4.68 ^a
CCH	5.6 $\pm$ 0.14 ^c	5.4 $\pm$ 0.04 ^a	28.5 $\pm$ 1.92 ^c	0.22 $\pm$ 0.04 ^c	53.1 $\pm$ 1.65 ^a
HS (control)	1.5 $\pm$ 0.33 ^f	4.2 $\pm$ 0.17 ^c	16.5 $\pm$ 3.04 ^d	1.6 $\pm$ 0.13 ^d	38.0 $\pm$ 4.56 ^b

Initial pH of the media was adjusted to 4.6 $\pm$ 0.1; BC, Bacterial cellulose; CCH, Coffee cherry husk extract (1:1 dilution); CSL, Corn steep liquor; EA, Ethanol (1.5%); AA, Acetic acid (1.0%); HS, Hestrin and Schramm medium

Results are average of 3 experiments ($n=3$) with standard deviation

Tensile strength and Elongation was calculated for 25 μ thickness ($n=10$) for comparison.

Values in column with similar letter are not significantly different ($P>0.05$).

of urea (0.2%) further reduced the production to 6.6 g/L. This may due to the inhibition of cell growth by the accumulation of residual acetate, as BC production is generally associated with cell growth (Matsuoka et al. 1996; Naritomi et al. 1998). Maximum BC (8.2 g/L) was found to be with 1:1 CCH and 8% CSL combination.

Properties of BC produced in CCH medium The final pH of all the combinations tried, ranged between 5.1–5.5 and the thickness of the films varied from 36.2–54.3 μ (Table 1). The thickness of the film varies based on the fibril thickness and the degree of compression (Velasco-Bedran and Lopez-Isunza 2007). Mechanical strength is an important parameter to check the quality of BC produced. BC produced using the standardized condition (1:1 CCH and 8% CSL combination) had a mechanical strength of 37.8 MPa tensile strength and 0.45 mm elongation. Among the experiments, BC film with better mechanical properties (42.4 MPa tensile strength and 0.58 mm elongation) was obtained with the combination of 1:1 CCH, 8% CSL, 1.5% ethanol and 1.0% acetic acid, even though this combination reduced the yield of BC. The tensile strength of BC

produced from HS media was estimated to be 16.5 MPa which was too low when compared with the BC obtained from CCH medium. The mechanical properties of the film are attributed not only to the increase in cross-sectional area that produces an increase in cross-sectional momentum, but also the increase in the number of 1,4 covalent bond (Keshk 2006). In the present study, the mechanical property increased when ethanol and acetic acid were added to the growth medium. Earlier workers have observed that the BC consists of highly entangled, randomly distributed cellulose microfibrils (McKenna et al. 2009). This random nature of deposition and extrusion results in an assortment of levels of aggregation and distances between physical cross-links (entanglements) for cellulose microfibrils. Further, research on the cross sectional momentum of BC produced from CCH, are required to understand the properties of these films.

The scanning electron micrograph of the films revealed it to be a fine net work of nanofibres with less than 1 μ m pore size. This is in accordance with the earlier observations of fibrellar nature of BC (George et al. 2008). Dilute alkaline solutions are capable of hydrolyzing and removing

Fig. 3 FTIR spectrum of bacterial cellulose produced from coffee cherry husk extract (CCH) and Hestrin and Schramm (HS) medium by *Gluconacetobacter hansenii* UAC09

impurities present in the cellulose pellicle including the embedded cells (George et al. 2005a). The weight loss in the pellicles as a result of alkaline treatment was found to be around 15–20%, which is attributed to the loss of protein and nucleic acid contents. The estimation of protein by both spectrophotometry and Lowry's method revealed that the native cellulose membrane comprises 14–18% of protein and 1–1.25% nucleic acids. Further analysis of the treated membranes had revealed the complete removal of proteins and nucleic acids. It is well known that proteins and deoxyribo nucleic acid can be hydrolyzed with alkaline solutions at boiling temperatures. As a result, it was found that the chemical treatments were effective for producing pure cellulose membranes (George et al. 2005a). The BC film treated with NaOH was devoid of cells and untreated film had cells with debris incorporated between fibrils (data not shown). FTIR spectrum of BC film produced in CCH extract was found to be similar to the control film with all the characteristic cellulose bands (Fig. 3). Earlier, while analyzing the chemical nature of the BC produced by the same organism, it was noticed that the BC produced had 1, 4 glycosidic bond with glucose monomers (Usha Rani et al. 2011).

Conclusion

The results obtained in this study revealed that an agro-waste such as coffee cherry husk can be used for the production of BC by *Gluconacetobacter* sp., which can bring down the cost of production. Such utilization of phytotoxic agro-waste containing more than 0.8% of polyphenols leads to the environmental management of high polyphenol containing organic waste. On one hand it provides cheaper alternate substrate for the production of value added product and on the other it helps to solve the disposal problem of toxic agro-waste. The BC yield achieved in this study was higher with the film had better mechanical properties, than the other commonly used synthetic medium.

Acknowledgements The first author wishes to acknowledge Council of Scientific and Industrial Research, New Delhi for awarding the Senior Research Fellowship. Authors are thankful to Director, Central Food Technological Research Institute, Mysore and to Head, Food Microbiology Department, for providing the facilities, for their support and help to carry out this study.

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