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Fermentative Production and Characterization of Carboxymethyl Bacterial Cellulose Using Date Syrup

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Abstract**—**In this study, static batch fermentation was used for bacterial cellulose production in date syrup solution (Bx. 10%) at 28°C using *Gluconacetobacter. xylinus* (PTCC 1734). The physicochemical properties of standard Sigma CMC and the produced carboxymethyl bacterial cellulose (CMBC) were studied using FT-IR spectroscopy, X-ray diffractometry (XRD) and Scanning Electron Microscopy (SEM). According to the FT-IR spectra the bands at 1664 and 1431 cm^{-1} indicate that carboxylic acid groups and carboxylate groups exist on the surface. The SEM imaging of CMBC and CMC carried out in magnification of 1K. Comparing the SEM imaging obviously showed that the ribbon shape in CMC remained but the length of ribbons became shorter while that shape changed to flake shape for CMBC. Determination of the area under XRD patterns demonstrated that the crystallinity amount of CMC was more than that for CMBC (51.08% and 81.84% for CMBC and CMC, respectively).

Keywords**—**Carboxymethyl bacterial cellulose, Fourier Transform Infrared spectroscopy, Scanning Electron Microscopy, X-ray diffractometry.

I. Introduction

CARBOXYMETHYLZ cellulose (CMC) or cellulose gum is a cellulose derivative with carboxymethyl groups (-CH2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone [4]. Bacterial cellulose (BC) has a wide range of potential applications including light transmitting optical fibers [1], a biological substrate medium [1, 11], food or food substitute [7], [5], [1], lint-free specialty clothing [1], optoelectronics devices [8], paper [5], [9], stereo diaphragms [5] and immobilization matrices of proteins or chromatography substances [5], [10].

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For BC to be suitable for diverse applications, some of its properties must be modified. The biologically degradable, regenerative raw material cellulose can be modified by chemical reactions in many ways [6]. Sodium carboxymethyl cellulose (CMC) is a polyelectrolyte which is formed when chloroacetic acid, or its sodium salt, reacts with alkali cellulose. Na-CMC is a copolymer of two units: β-D-glucose and β-D-glucopyranose 2-O-(carboxymethyl)-monosodium salt, not randomly distributed along the macromolecule, which are linked via β-1,4-glycosidic bonds [2]. CMC is widely used as a thickener, water binder, extrusion aid and film former in pharmacy, cosmetics and the food industry in order to improve the consistency and flow properties [3], [6]. In this study, carboxymethyl cellulose was produced from bacterial cellulose. The chemical modification was characterized by Fourier Transform Infrared Spectroscopy (FT-IR). Changes in the physical properties were observed using scanning electron microscopy (SEM) and X-ray diffractometry (XRD).

II. Materials and Methods

A. Preparation of date syrup

The initial extract was prepared by soaking 200 g of stone free, low quality date fruits in 500 ml distilled water then blended in a blender for 1 min at low speed, and for further 3 min at high speed. The homogenized extract was filtered through a double layer of cheese cloth. The homogenized extract was filtered through a double layer of cheese cloth. The residue was then washed with hot water and solution made up to the volume required for a concentration of 20 %; subsequent dilutions to brix 10 were made with distilled water. To remove all insoluble solids, the date extract solutions were then centrifuged (Sorvall, RC-5; USA) at 10000×g for 30 min at room temperature.

B. Stock Culture

The organism exploited for the production of cellulose in this work was a strain of *Gluconacetobacter xylinus*. *G. xylinus* was obtained from the Persian Type Culture Collection (PTCC) strain number 1734. The microorganisms were maintained in the test tubes containing tomato serum medium and were transferred monthly. The typical composition of the stock culture medium was 50 g/l glucose, 5 g/l bactopeptone, 5 g/l yeast extract and 10% by volume tomato juice at pH, 6.8. The stock cultures were stored at 5˚ C to slow down growth and cellulose production. The rate of

cellulose production in the stock culture test tubes was regularly checked to ensure that mutation had not taken place.

C. *Inoculum Preparation*

A culture medium composed of 5% wt. glucose, 0.5% bactopeptone, 0.5% yeast extract, 0.27% sodium phosphate monobasic and 0.12% citric acid was used in all cellulose production experiments.

About 200 ml of this medium contained in 500 ml Erlenmeyer flasks was autoclaved at 121˚C for 15 minutes prior to inoculation. After cooling to room temperature, the flasks were inoculated with the stock culture and incubated in a Benmarin shaker (240 rpm) set at 28 ˚C at pH, 6.8. The organisms were collected by centrifugation (2500×g, 20 min) and resuspended in liquid medium and the bacteria were inoculated into liquid fermentation media in Erlenmeyer flasks containing date syrup (Brix 10) as a carbon source. The fermentation medium component is shown in Table I.

TABLE I
THE COMPONENT OF THE FERMENTATION MEDIA

Components	Quantity
Date syrup (Brix 10)	1 L
Na_2HPO_4	2.7 g
Bacto peptone	5 g
Yeast extract	5 g
Citric acid	1.5 g

D.Pellicle production and purification

Bacterial cellulose was produced by *G. xylinus* in 2000 ml Erlenmeyer flask at 28 ˚C for 30 days. After incubation, the pellicle produced on the surface of media containing date syrup was separated into the supernatant and pellicles by centrifugation. For removal of microbial product contaminants, the pellicles were washed with water, 4 % (w/v) sodium hydroxide solution in boiling bath (30 min), 6 % (v/v) acetic acid and then water, successively.

E. Production of carboxymethyl bacterial cellulose

The procedure for production of CMBC is shown in Figure 1. Twenty grams of cellulose (wet basis) from bacterial cellulose, 100 ml of isobutyl alcohol and 20 ml of NaOH having concentration of 30% were mechanically stirred for 90 min in an Erlenmeyer flask. Reaction occurred in a Benmarin shaker (250 rpm) set at 25 ˚C. The mixture was filtered and then 5.0 g of sodium chloroacetate were added and shaking continued at 70 ˚C for 360 min. After the reaction was completed, the mixture was neutralized with acetic acid (90%) and filtered.

Cake was purified by washing with methanol 70% to remove undesired salts, filtered and dried at 70 ˚C.

F. *Fourier Transform Infrared Spectroscopy (FT-IR)*

The samples (CMBC, CMC, BC and cellulose) were well mixed with potassium bromide (KBr) powder and pressed into a small tablet. FT-IR spectra were recorded using a Brucker spectrometer (EQUINOX55, Germany) in the transmittance mode with a resolution of 1 cm^{-1} in the range of 4000-400 cm^{-1}.

G. Scanning electron microscopy (SEM)

Thin layers of samples were coated with gold using an ion sputter (Fisons Instruments, UK). The coated samples were viewed and photographed using the scanning electron microscope (model 5526, Cambridge, UK) at 20 kv.

H. X-Ray analysis:

To determine the crystallinity of CMBC and CMC, the X-ray diffraction (XRD) patterns of the samples were collected on a Siemens (D5000-Germany) Standard Theta/2Theta diffractometer using a copper X-ray source. Scans were collected at 2 degree per minute from 5–60 degree 2θ. The samples were lyophilized overnight using a lab-scale freeze drier (Armfield model) and pressed into a thin and flat layer (~1.5 mm thickness) for analysis.

III. Results and Discussion

A. FT-IR Spectroscopy

Figure 2-a shows the FT-IR spectrum of the pure cellulose. The bands at 1664 and 1431 cm^{-1} indicate that carboxylic acid groups and carboxylate groups exist on the surface. The band of 2999 cm^{-1} is attributed to CH2 stretching. The band at 1058 cm^{-1} could be associated with ether C-O-C functionalities. The band at 3415 cm^{-1} is attributed to the presence of hydroxyl groups (-OH). Figure 2-b, c, d shows the FT-IR spectra of bacterial cellulose, carboxymethyl bacterial cellulose and carboxy methyl cellulose, respectively. According to the FT-IR spectra (Fig. 2), the bands at 1664 and 1431 cm^{-1} indicate carboxylic acid groups and carboxylate groups exist on the surface which this state confirms the production of CMBC from the initial BC.

B. SEM

Figure 3 shows the morphological structures of CMBC and CMC from lyophilized samples. Figures clearly show that the conversion of BC to CMBC lead to change in its ribbon shape and make it as a flake or sheet shape. Comparing Fig. 3-c with 3-d obviously showed that the ribbon shape in CMC remained but the length of ribbons became shorter. The figure revealed that the CMBC produced from bacterial cellulose which was produced in date syrup fermentation media by *G.xylinus* appeared coarser and less entangled than CMC

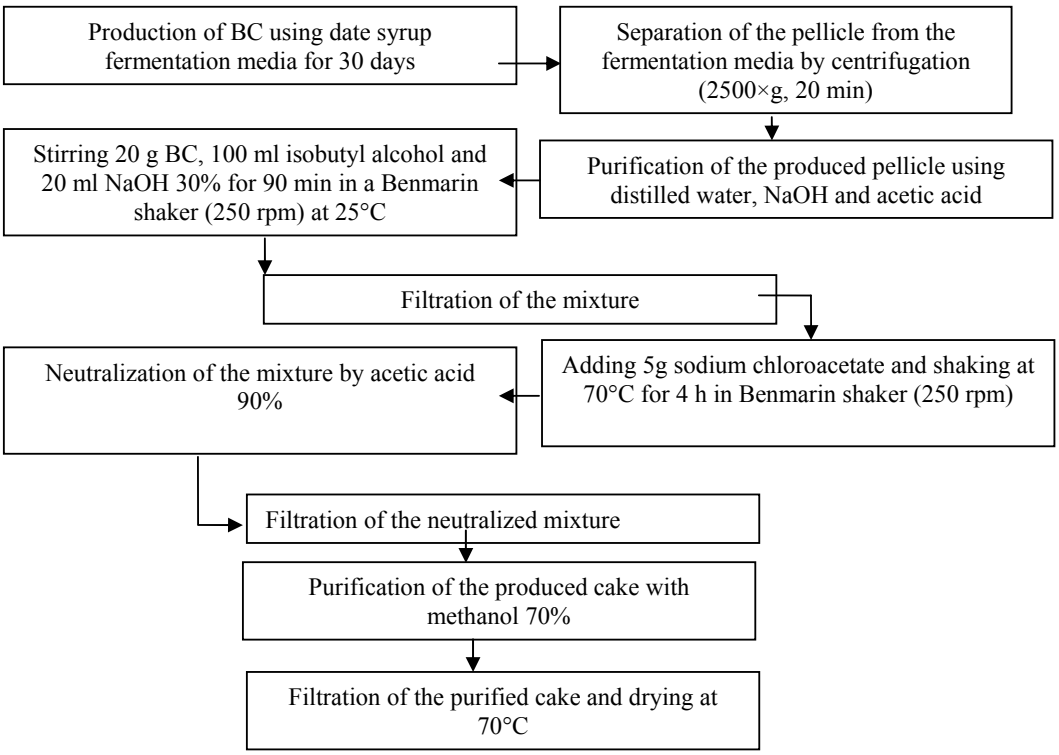

Fig. 1 Schematic diagram of the procedure for CMBC production.

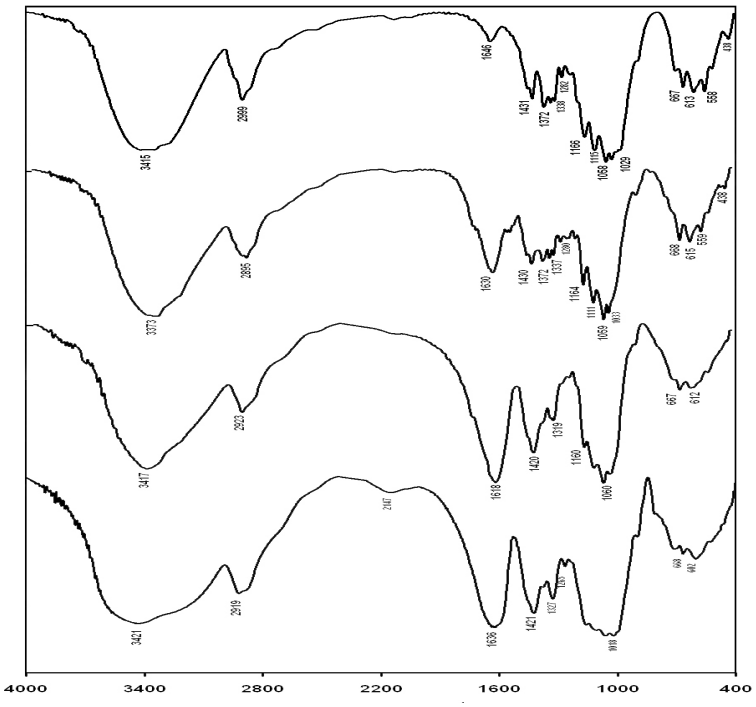

Fig .2 FT-IR spectra of (a) pure cellulose, (b) bacterial cellulose and (c) carboxymethyl bacterial cellulose and (d) carboxymethyl cellulose.

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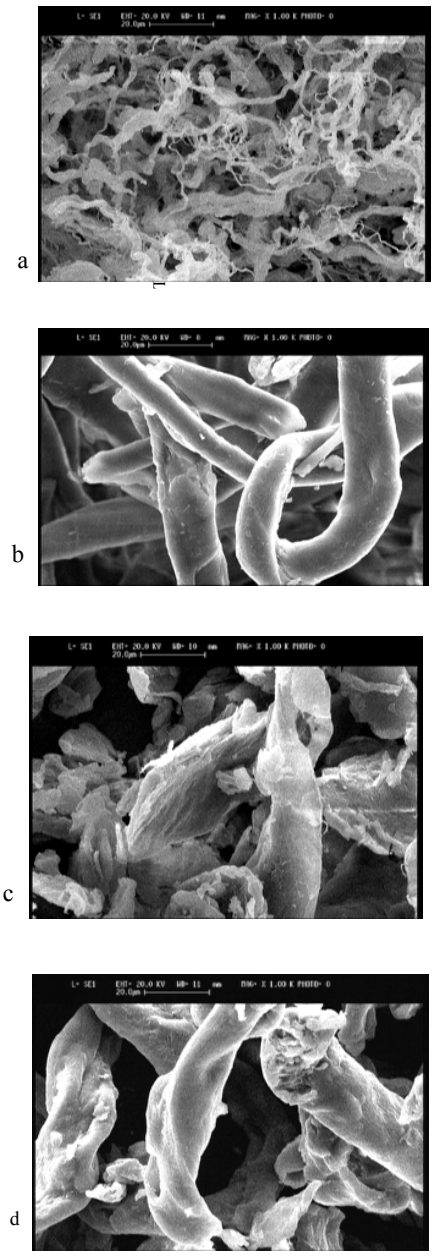

Fig. 3 SEM images of (a) BC, (b) cellulose, (c) CMBC and (d) standard CMC.

C. X-Ray diffractometry analysis

Figure 4 Shows XRD patterns of the samples. The area under XRD patterns is the measure of samples crystallinity which was 51.08% and 81.84% for CMBC and CMC, respectively. Less crystallinity of CMBC than CMC makes it chemically a reactive component when participates in a chemical reaction. Since CMC and CMBC consists of long, fairly rigid molecules that bear a negative charge due to numerous ionized carboxyl groups (according to the FT-IR results that is shown in Figure 2-c,d), electrostatic repulsion causes their molecules in solution to be extended. Furthermore, adjacent chains repel each other [4]. Consequently, CMC and CMBC solutions tend to be both highly viscous and stable.

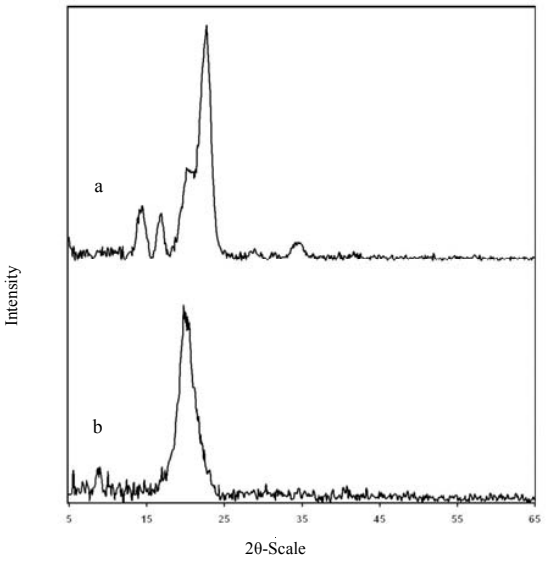

Fig. 4 XRD patterns of (a) CMBC and (b) CMC.

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