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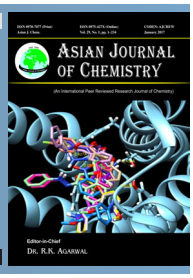

Production and Optimization of Bacterial Cellulose with Different Carbon and Nitrogen Sources Using *Gluconacetobacter xylinus* C18 Strain

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Cellulose is eco-friendly homopolysacharide biomacromolecules which are produced by plants and several genera of bacteria. Bacterial cellulose has been biosynthesized mainly by Gram-negative bacteria such as *Acetobacter, Pseudomonas, Salmonella, Alcaligenes, Azotobacter, Rhizobium etc.,* though Gram-positive bacteria *Sarcina ventriculi* has also been separated for its biosynthesis. Bacterial cellulose having a great potential of it industrial application due to its unique properties such as high purity, high crystallinity, hydrophilicity, elasticity, high water holding capacity, higher degree of polymerization and, good mechanical strength. The present studies focus to evaluate the potential of *Gluconacetobacter xylinus* C18 at different carbon and nitrogen sources in different condition such as temperature, pH and incubation time. The new strain C18 produced the highest 4.12 g/L of cellulose when we used (2 % w/v) glucose as carbon source and (1 % w/v) yeast extract as nitrogen source were taken. The pH and temperature were maintained as 6.5 and 30 °C, respectively. The bacterial cellulose was characterizationed by FT-IR and scanning electron microscopy.

Keywords: Nitrogen source, Bacterial cellulose, *Gluconacetobacter xylinus*.

INTRODUCTION

Cellulose is a homopolysaccharides biopolymer having the chemical formula $(C_6H_{10}O_5)_n$, which is consisting of a linear chain of several hundred to many thousands of β(1→4) linked D-glucose units. Cellulose is the most abundant macromolecule on earth that is mostly produced by green plants and algae [1]. Some species of bacteria also produce the cellulose that is the alternate source of cellulose production to reduce the demand of plant cellulose [2]. Bacterial cellulose is produced by several species of Gram-negative bacteria, such as *Acetobacter, Pseudomonas, Salmonella Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina* and *Salmonella.* Some Gram-positive bacteria also produce the cellulose such as *Sarcina ventriculi* [3]. Production of cellulose from *Acetobacter xylinum* was first reported by Brown [4]. Now it is known as *Gluconacetobacter xylinus. Gluconacetobacter xylinus* is a most efficient producer of cellulose. They are rode shape, Gram-negative, non-pathogenic, aerobic bacteria which synthesized the cellulose in the form of microfibril and form a thick mat in the interface of fermentation medium [5,6]. Bacterial cellulose had many advantage over plant cellulose due to its unique properties; high purity, high crystallinity, hydrophilicity, high degree of polymerization [7-9]. Bacterial cellulose also has the higher water holding capacity and tensile strength. Due to these properties bacterial cellulose has been used in many application such as pharmaceutical industries as wound dressing, paper making, healthcare, cosmetics, artificial blood vessel, artificial skin and coating, binding, thickening, emulsifying and Nata de coco, as dietary fiber in food industries [10-12].

EXPERIMENTAL

All chemicals used for medium and analytical work such as sucrose, glucose, fructose, maltose, manitol, galactose, glycerol, yeast extract, peptone ammonium nitrate, urea, thiourea, citric acid, disodium phosphate, *etc*. were purchased from Merck (India), Hi-media and Ranbaxy.

Maintenance of bacterial culture: Bacterial culture were maintained on growth media containing yeast extract (5 g/L), peptone (5 g/L), mannitol (25 g/L). 50 mL of liquid medium were taken into 250 mL Erlenmeyer flask and autoclaved at 121 °C for 20 min. Sterilized medium was inoculated with newly isolated *Gluconacetobacter xylinus* C18 and incubated at 30 °C for 48 h.

Preparation of fermentation media: Hestrin and Schramm (HS) medium was prepared with composition glucose (20 g/L), yeast extract (5 g/L), peptone (5 g/L), citric acid (2.7 g/L),

disodium hydrogen phosphate (1.17 g/L) [13]. The (2 % w/v) different carbon sources [glucose, fructose, maltose, sucrose, galactose, manitol and glycerol and (1 % w/v) nitrogen sources] were tested for bacterial cellulose production. The 1 % (w/v) different types of nitrogen sources (peptone, yeast extract, tryptone, beef extract, urea, thiourea and ammonium nitrate) were taken for bacterial cellulose production. The pH of medium was adjusted to 6.5. The fermentation medium was sterilized at 121 °C for 20 min. The 2 % inoculums of *Gluconacetobacter xylinus* C18 was added into a flask aseptically and incubated at 30 °C for 192 h.

Isolation and purification of bacterial cellulose: The cellulose pellicle produced during the fermentation was harvested and isolated by centrifugation at 5000 rpm. After centrifugation the biomass was washed with distilled or deionized water to removed sugar residues. The washed biomass boiled with 2 % (w/v) NaOH solution for 20 min to remove cells from the cellulose matrix [14]. The treated bacterial cellulose neutralized by deionized water until the remaining alkali is removed. The wet and dry weight of cellulose was measured [15-17].

Optimization of media components and process parameters: The different carbon and nitrogen sources were tested for bacterial cellulose production. The % sugar utilization is also calculated by standard methods. The process parameter pH varied from 3.5 to 8.5, temperature range 20-40 °C and incubation period from 48 to 240 h. has been standardized for maximum bacterial cellulose production.

Characterization of bacterial cellulose: The surface properties of bacterial cellulose were characterized by Fourier transforms infrared and scanning electron microscopy.

RESULTS AND DISCUSSION

Effect of different carbon sources on bacterial cellulose production: Effects of nitrogen sources on cellulose production were observed in Fig. 1. The maximum bacterial cellulose (4.18 g/L) was observed in glucose followed by manitol (3.96 g/L), sucrose (3.85 g/L) and fructose (3.52 g/L).The carbon is a sole source for cellulose production and cell growth.

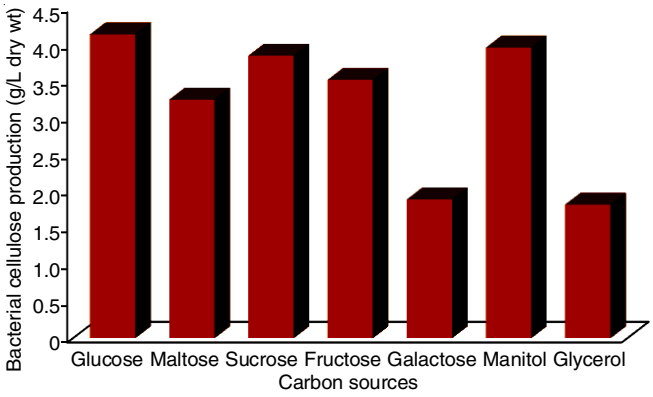

Fig. 1. Effect of different carbon sources on bacterial cellulose production

Effect of nitrogen sources for bacterial cellulose production: Effects of nitrogen sources on cellulose production were observed in Fig. 2. The nitrogen source is an important factor for bacterial cell growth. The yeast extract was found the most effective nitrogen source for bacterial cellulose production.

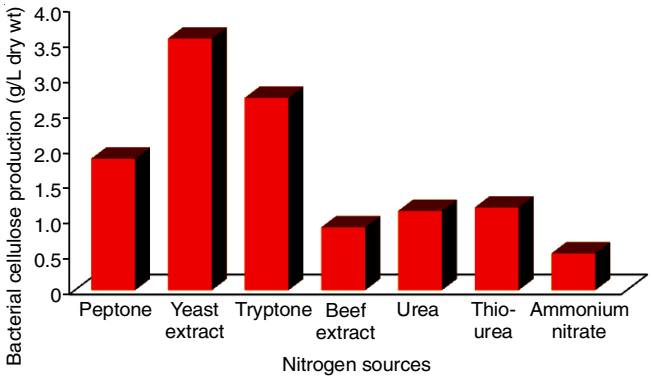

Fig. 2. Effect of nitrogen sources for bacterial cellulose production

The maximum bacterial cellulose was observed (3.56 g/L) in yeast extract followed by tryptone (2.75 g/L), peptone (1.86 g/L) and thiourea (1.17 g/L). This may be due to that yeast extract is rich in amino acid and vitamin B complex which provide the extra nutrient to bacteria [18].

Sugar utilization for bacterial cellulose production: The maximum sugar utilized 98 and 94 % by the *Gluconacetobacter xylinum* C18 when we used glucose and manitol as sole carbon sources followed by maltose, sucrose and fructose (Fig. 3). The similar result has been observed by Panesar *et al.* [19].

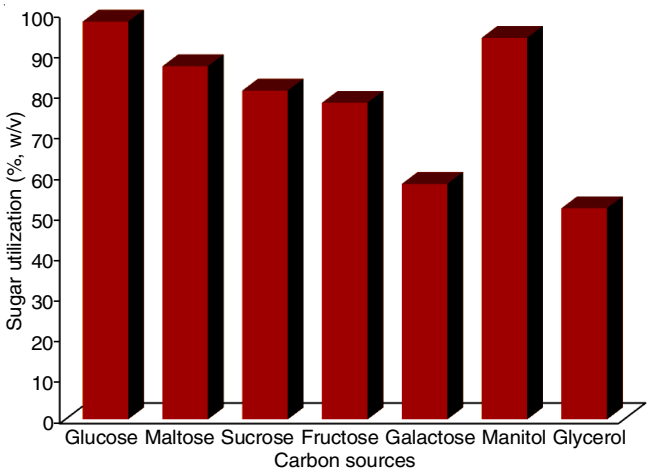

Fig. 3. (%) Sugar utilization for bacterial cellulose production

Effect of pH on bacterial cellulose production: The cellulose producing bacteria was tested at different pH ranging 4.5 to 8.5. The bacterial cellulose was observed in all pH range 4.5 to 8.5. The maximum bacterial cellulose was observed at pH 6.5 (Fig. 4). This may be due to the fact that the optimum pH required for oxidative reductive reaction and also influenced the physiology of bacteria by affecting the nutrient solubility uptake [20]. Several workers [21-23] reported the minimum cellulose production at pH 4 and highest at pH 7. The pH plays an important role in the growth of bacteria and cellulose production.

Effect of temperature on bacterial cellulose production: The temperature range (20, 25, 30, 35, 37 and 40 °C) was examined. The bacterial cellulose production was observed at all range but the maximum bacterial cellulose (3.92 g/L) was observed at 30 °C (Fig. 5). Similar results have been reported

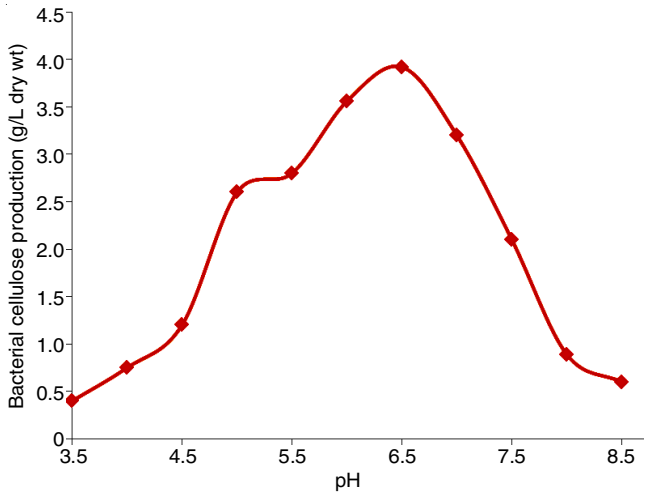

Fig. 4. Effect of pH on bacterial cellulose production

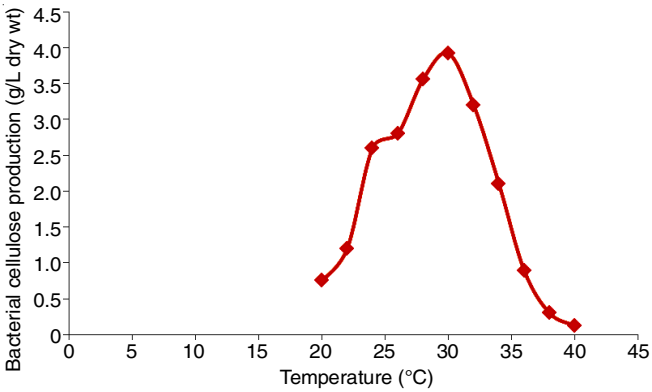

Fig. 5. Effect of temperature on bacterial cellulose production

[22,24-26]. The optimum ranges of temperature for bacterial cellulose were 28 to 30 °C [14]. The bacterial cellulose production was not observed at 40 °C. This might be due to a number of enzymes and complexes takes part in the carbon metabolism pathway of cellulose synthesis which is work at specific temperature [10].

Effect of incubation time on bacterial cellulose production: The effect of incubation time indicated that with increase in incubation time the cellulose production increased up to 192 h. After 192 h the small decline was observed in Fig. 6. The maximum bacterial cellulose was observed at 192 h. The bacterial cellulose production was observed after 48 h.

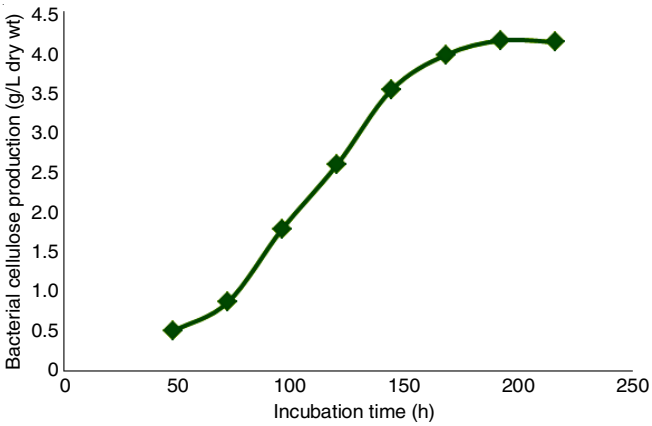

Fig. 6. Effect of incubation time on bacterial cellulose production

The similar trends were previously reported by Panesar *et al.* [19] and Bae & Shoda [27].

Morphology and characterization of bacterial cellulose: The spectrum of bacterial cellulose was examined by FTIR-ATR methods. The IR spectra revealed the characteristics absorption bandof bacteria cellulose. The IR spectrum were showed the strong band in the region of 3649-3349 cm^{-1} of OH stretching (hydroxyl functional group) [7,28] and in the range of 1648-1447 cm^{-1} showed the strong band of C=O (carbonyl group). The several typical bands for bacterial cellulose were shown in the region of 1716-1696 cm^{-1}. Due to C-O and C-O-C asymmetric stretching and symmetric stretching were shown in the region of 1057-1030 cm^{-1} for bacterial cellulose [29,31]. The microfibrilar nano-sized structure was observed in SEM. The random microfibrilar interwoven network was seen [27,30,32]. The SEM image confirms the bacterial cellulose exhibit the rounder fibrils structure network.

Conclusion

The *Gluconacetobacter xylinus* C18 isolate was tested for bacterial cellulose production. The different carbon and nitrogen sources were screened for maximum cellulose production. The (2 % w/v) glucose was found the best source as a carbon and (1 % w/v) yeast extract as a nitrogen source for maximum bacterial cellulose production. The maximum sugar utilization was found 98 % with glucose and 94 % with manitol. The best pH temperature for maximum bacterial cellulose production was 6.5 and 30 °C. The maximum bacterial cellulose (4.12 g/L) production was observed at 192 h. IR spectrum showed typical bands due to presence of –OH group, ether linkage and pyranose ring confirm that the produced material to be bacterial cellulose. The SEM images show the microfibril structure of bacterial cellulose with high porosity.

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