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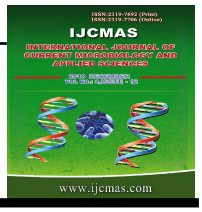

Original Research Article

Production of Microbial Cellulose from the New Bacterial Strain Isolated From Temple Wash Waters

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A B S T R A C T

Cellulose is the most abundant natural biopolymer on the earth, synthesized by plants, algae and also some species of bacteria. It is also produced by some animals (e.g., tunicates). Unlike cellulose from plants, bacterial cellulose (BC) is chemically pure and free of lignin and hemi-cellulose. BC has high crystallinity and a high degree of polymerization. Plant-derived cellulose and BC have the same chemical composition but different structures and physical properties. Microbial cellulose is an exopolysaccharide produced by various species of bacteria, such as those of the genera *Gluconacetobacter* (formerly *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, and *Salmonella* (Chawla *et al.*, 2009). Bacterial cellulose has important applications in a variety of food formulations (Khan *et al.*, 2007). It is especially used when low-level use, lack of flavor interactions, foam stabilization, and stability over a wide range of pH, temperature, and freeze-thaw conditions are required. Potential uses include pourable and spoonable dressings, sauces, and gravies; frostings and icings; sour cream and cultured dairy products; whipped toppings and aerated desserts, and frozen dairy products (Chawla *et al.*, 2009 ; Khan *et al.*, 2007). Microbial cellulose promises to have many new applications in wound care that extend beyond burn applications including, but not limited to, the following: surgical wounds, bedsores, ulcers, tissue and organ engineering. The objective of this work was to produce low cost environmentally friendly cellulose. Cellulose producing bacteria was isolated from the temple wash water and were identified and characterized by microbiological methods. The production parameters were optimized and laboratory scale cellulose was produced, purified and quantified. The yield was found to be 2.93 ± 0.01 g/L under laboratory conditions

K e y w o r d s

Bacteria; Cellulose; *Gluconaceto-bacter*; *Rhizobium*, *Sarcina* Optimization.

Introduction

Exopolysaccharides are long chain polysaccharides which are composed of branched, repeating units of sugars or sugar derivatives, such as glucose, galactose and rhamnose in different amounts. They are classified into two

groups: homopolysaccharides (cellulose, dextran, mutan, pullulan, curdlan), and heteropolysaccharides (gellan and xanthan) (Chawla *et al.,* 2009). Cellulose is the most abundant macromolecule on earth (Zhao *et al.,* 2007) that is mostly produced by vascular plants. Cellulose is an unbranched polymer of β-1→4-linked glucopyranose residues. Cellulose is the most abundant natural biopolymer on the earth, synthesized by plants, algae and also some species of bacteria. A substitute to reduce the demand from plants is the production of cellulose from another resource, such as the use of a microbial system (Brown , 2004).

Bacterial cellulose is preferred over the plant cellulose as it can be obtained in higher purity and exhibits a higher degree of polymerization and crystallinity index. It also has higher tensile strength and water holding capacity than that of plant cellulose, making it more suitable raw material for producing high fidelity acoustic speakers, high quality paper and dessert foods (Shoda and Sugano , 2005). Fibrils of bacterial cellulose are about 100 times thinner than that of plant cellulose, making it a highly porous material, which allows transfer of antibiotics or other medicines into the wound while at the same time serving as an efficient physical barrier against any external infection. The molecular formula of bacterial cellulose $(C_6H_{10}O_5)_n$ is the same as that of plant cellulose, but their physical and chemical features are different (Brown , 1886). The BC network structure comprises cellulose nanofibrils 3-8 nm in diameter, and the crystalline regions are normal cellulose I. The specific properties such as the nanometric structure, unique physical and mechanical properties together with its higher purity have lead to a great number of commercial products (Klemm *et al.,* 2001; Klemm *et al.,* 2005 ; Czaja *et al.,* 2006).

Microbial cellulose is produced by various species of bacteria, such as those of the genera *Gluconacetobacter* (formerly *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, and *Salmonella* (Shoda and Sugano, 2005). Production of cellulose from *Acetobacter xylinum* was first reported by A.J.Brown (1886).

Gram-negative species like *Acetobacter, Agrobacterium, Achromobacter, Aerobacter, Sarcina, Azotobacter, Rhizobium, Pseudomonas, Salmonella* and *Alcaligenes* produce cellulose. Cellulose is also synthesized by the Gram-positive bacterium *Sarcina ventriculi*, accounting for about 15 % of the total dry cell mass. The most effective producers of cellulose are *A. xylinum* , *A. hansenii* and *A. pasteurianus* (Bellamy , 1974; . Brown Jr 1987; Gromet-Elhanan and Hestrin, 1963; Geyer *et al.,* 1994; Geyer *et al.,* 1994b; Jung *et al.,* 2005; Park, *et al.,* 2003 ; Yoshino *et al.,* 1996).

Acetobacter xylinum produces two forms of cellulose: i) cellulose 1, the ribbon like polymer, and ii) cellulose 2, the thermodynamically more stable amorphous polymer (Yu and Atalla, 1996).

Bacterial cellulose is used as a diet food and to produce new materials for high performance speaker diaphragms, medical pads and artificial skin. It is also used extensively in wound healing (Chawla *et al.,* 2009).

Relatively high cost of the production of cellulose may limit its application to high value-added products as well as specialty

chemicals (Legge , 1990). Significant cost reductions are possible with improvements in fermentation fermentation efficiency and economics of scale, the lower limit of the cost of microbial cellulose being determined by the price of the raw material substrates.

A. xylinum has been applied as model microorganism for basic and applied studies on cellulose. It is most commonly studied source of bacterial cellulose because of its ability to produce relatively high levels of polymer from a wide range of carbon and nitrogen sources. It is a rod-shaped, aerobic, Gram-negative bacterium that produces cellulose in the form of interwoven extracellular ribbons as part of primary metabolite. This bacterium grows and produces cellulose from a wide variety of substrates and is devoid of cellulase activity (Bielecki *et al.,* 2005 ; Puri, 1984). The study involves the isolation and identification of cellulose producing bacteria from the temple wash waters and also the optimization and production of bacterial cellulose from the new isolate.

Materials and Methods

Screening of Microorganisms

In India, almost in all parts, coconut is being offered as part of religious practice in temples. The coconuts will be broken and the water will be poured on the deity, then the water is made to go out of the sanctum sanctorum and get collected in small sump and from there it will be made to go out of the temple for discharge purpose. The sump is a potential source for various kinds of microorganisms.

The wash waters from the sump are collected in a sterile conical flask and transported to the laboratory for further processing. The sample was applied on/in the culture media by pour plate and spread plate methods. The plates were incubated at 30°C for 48 hours and screened for cellulose production.

Identification and characterization

The isolated bacteria was identified and characterized based on the biochemical, morphological and cultural tests, the isolate was identified as *Acetobacter* sp.DR-1. The isolate was maintained on agar slants and used for further works by sub-culturing.

Culture media for Cellulose production

The composition of medium was (g/L): glucose (20), yeast extract (5), peptone (5), citric acid (2.7), disodium hydrogen phosphate (1.17). The pH of medium was initially adjusted to 5.5, and fermentation medium was sterilized. *Acetobacter* sp.DR-1 cells taken from the seed culture and was added into a flask (2% inoculum) and were incubated at 28°C for 10 days (Panesar *et al.,* 2009).

Detection and Quantification of cellulose produced

The bacterial cellulose produced during the course of the fermentation was measured at the end of each run. At the end of the 5-day incubation, the culture broth was centrifuged at 3300 × g for 20 min . For biomass, the precipitated pellicle was added to 90 ml of buffer (0.1 M potassium acetate-acetate buffer (pH 5.0) and 10 ml of 20% cellulase and incubated at 50°C with shaking at 100 rpm for 1 h to hydrolyze BC (Kouda *et al.,* 1997). Then, the solution was centrifuged at 3,300 × g for 20 min. The precipitate was dried in an oven at 80°C overnight and then weighed

to determine biomass. For BC determination, the precipitated BC pellicle was treated with 0.1 N NaOH solutions at 80°C for 30 min to remove the bacterial cells and medium components (Hwang *et al.,* 1995). This NaOH treatment was repeated three times and then, the solution was centrifuged at 3,300 × g for 20 min. The obtained cellulose was dried in an oven at 80°C overnight and then weighed. The dried bacterial cellulose samples were then weighed, and values were reported as mg/100 ml of the original medium.

Carbon sources

To study the effect of different carbon sources on the cellulose production, the following carbohydrates were added to the above culture media at 1% concentration: glucose, sucrose, lactose, maltose and Mannitol.

Nitrogen sources

Peptone, Urea, Ammonium sulphate and sodium nitrate were the nitrogen sources which were screened for their ability to support cellulose production by *Acetobacter* sp. DR-1. They were added at 0.5% concentration to the culture media.

Temperature

The effect of various incubation temperatures, like 25°C, 30°C, 35°C, 40°C and 50°C, on the cellulose production by *Acetobacter* sp. DR-1 was studied by incubation the flasks at above temperatures.

pH

The effect of different pH's on cellulose production by *Acetobacter* sp. DR-1 was studied by maintain the culture media pH at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 and then incubating them at 30°C for 1 week.

Shake flask / still culture

The effect of aeration on cellulose production was tested by still culture and shake flask method. The procedure was to incubate inoculated culture flasks at stationary condition and at 40 rpm in shaker incubator respectively. The flasks were tested for cellulose production.

Optimization

The production of cellulose under optimized conditions was also carried out and the quantity of cellulose produced was compared with the cellulose produced under un-optimized condition.

Sugarcane juice

Sugar cane juice was procured from local market and was filter sterilized using micro-filter (with pore size being 0.22 µm). 100ml of sugar cane juice was taken in a 250ml sterile conical flask and was inoculated with the pure culture of *Acetobacter* sp. DR-1. The flask was incubated at 30°C C for 7 days and sampling and assay was done after every 6 hours.

Comparison of cellulose production by the new strain with the standard one

A comparison of cellulose producing capability of *Acetobacter* sp. DR-1 with that of standard cellulose producing bacteria, like *Acetobacter aceti* ATCC 23747 and *Acetobacter xylinum* ATCC 11142 supplied by IMTECH, Chandigarh and NCIM, Pune, India, respectively, was also carried out.

Statistical analysis

All treatments were replicated at least two times. The significant difference of the results was evaluated using the Generalized Linear Model (GLM; with $p < 0.05$) at 95% confidence limit using SPSS software.

Results and Discussion

Isolation and Identification of cellulose producing bacteria

The isolated bacterial species were identified by their cultural and biochemical characteristics. Based on the result the isolate was identified as *Acetobacter* sp. DR-1. The results are shown in the table-1.

The isolate was gram-negative rods occurring as individuals, pairs, chains or small clusters. It was motile, and obligate aerobe which metabolizes primarily glucose which it uses in cellulose synthesis. The colonies were circular, convex, regular shape and 3 mm diameter. The cells were Short rods/cocco-bacilli in shape and Mono and diplo-bacilli in arrangement under microscope.

T. T. Kadere *et al.,* (2008) investigated the occurrence and identification of the dominant spoilage genera of acetic acid bacteria in coconut wine (mnazi), by plating the dilution series previously pre-enriched in a basal medium onto GYP agar, followed by physiological and biochemical tests. Both *Acetobacter* and *Gluconobacter* strains were isolated and identified by them (Kadere *et al.,* 2008).

Similarly, five strains of acetic acid bacteria were isolated from wastes of vinegar fermentation by Y. Andelib Aydin and Nuran Deveci Aksoy (2009). Three of the strains were found to secrete cellulose. These strains were identified by several biochemical and physiological tests and the results were compared with refererence strains *Gluconacetobacter xylinus*DSM 46604 and *Gluconacetobacter hansenii* DSM 5602 (Andelib and Nuran,2009).

Effect of carbon sources on cellulose production

Mannitol was found to be the best carbon source as it supported the maximum cellulose production viz., 1.38g/L. the results are given in table-2 and figure-1.

Usually glucose and sucrose are used as carbon sources for cellulose production although other carbohydrates such as fructose, maltose, xylose, starch and glycerol have also been tried. The effect of initial glucose concentration on cellulose production is also important since the formation of gluconic acid as a byproduct in the medium decreases the production of cellulose. Cellulose yields at initial glucose concentration of 6, 12, 24 and 48 g/L were studied and the consumption of glucose was found to be 100, 100, 68 and 28% of the initial concentration respectively (Masaoka *et al.,* 1993).

Fructose was found to be the best carbon source at 2% concentration for the production of bacterial cellulose by S.Changjin *et al.,* (2002). The production of bacterial cellulose (BC) using *Gluconacetobacter xylinus* (=*Acetobacter xylinum*) ATCC 10245 from three categories of carbon sources (Monosaccharide, disaccharide and alcohol) was examined. Glycerol gave the highest yield, followed by glucose, fructose, inositol and saccharose. The highest production efficiency of glycerol

might be due to the rate of its consumption (45.4%), which is lower than that of glucose (97%). The carbon sources and the cellulose yield supported are as given: Glucose (8.4 %), Fructose (7.9%), Inositol (7.4%) Glycerol (13.0%) (Sherif *et al.*, 2005).

Effect of nitrogen sources on cellulose production

Peptone supported the maximum cellulose yield (0.86 g/L), when compared to the rest of the nitrogen sources. The results are shown in the table-3 and figure-2.

Nitrogen is a main component of proteins necessary in cell metabolism, and comprises 8-14% of the dry cell mass of bacteria. The effect of various nitrogen sources on the production of bacterial cellulose has been reported; casein hydrolyzate gave yield of 4.8 g/L of cellulose production (Matsuoka *et al.*, 1996). The addition of extra nitrogen favors the biomass production but diminishes cellulose production (Masaoka *et al.*, 1993).

Effect of incubation temperature on cellulose production

30°C was found to be the best temperature to produce maximum yield of cellulose by the new isolate. It supported 0.88 g/L of cellulose. The results are given in the table-4 and figure-3.

Temperature is a crucial parameter that affects both growth and cellulose production. In the majority of experiments, the maximal cellulose production was observed between 28 and 30°C (Hestrin and Schramm, 1954). *Acetobacter pasteurianus* HBB6 and *Acetobacter lovaniensis* HBB5 strains, were isolated from vinegar in Turkey and grown on HS (Hestrin-Scharmm) medium and beet molasses and then the effect of incubation temperatures (4, 22, 30 and 37°C) were examined by Esin Poyrazoglu Çoban and Halil Biyik (2011). It was found that the optimum temperature was obtained at 30°C; the lowest yield was obtained at 22°C in HS medium and beet molasses medium, there was no cell growth and cellulose production at the temperature of 4°C in HS medium and beet molasses (Esin Poyrazoglu Çoban and Halil Biyik , 2011).

Studies revealed that the optimal growth temperature for cellulose production is 25 to 30°C (Cannon and Anderson, 1991). Most authors used the temperature range from 28 to 30°C. The variation in temperature caused changes of cellulose degree of polymerisation and water-binding capacity, whereas, the bacterial cellulose synthesized at 30°C had a lower DP (~10,000) and a higher water binding capacity (164%) in comparison to that produced at 25 and 35°C (Esin Poyrazoglu Çoban and Halil Biyik , 2011).

Jonas and Farah (1998) stated that the optimal growth temperature for cellulose production is 25 to 30°C, although most researchers used 28 to 30°C (Jonas and Farah , 1998). Son *et al.*, (2001) examined the effects of various temperatures (20 to 40°C) using HS medium. The optimum temperature for cellulose production was observed at 30°C. There was no significant difference in cellulose production at 25°C. However, cellulose production decreased above 35°C (Son *et al.*, 2003). According to Pourramezan *et al.*, (2009), the optimum temperature was obtained at 30°C and the lowest yield was obtained at 45°C (Pourramezan *et al.*, 2009).

Table.1 Identification of *Acetobacter* sp.

Test(s)	Result(s)
Oxidation of	
D- Fructose	+/-
D-Glucose	+
Glycerol	+
D-Mannitol	+
Lactose	+
Sucrose	+
Growth on	
Ethanol	+
D-xylose	+
D-Galactose	+
Glycerol	+
Dulcitol	-
Lactose	+
Maltose	+
Sucrose	+
D-Glucose	+
Acid Formation from	
D-Xylose	+
D-Galactose	+
Glycerol	+
Dulcitol	-
Lactose	+
Maltose	+
Sucrose	+
D-Glucose	+
Oxidation of	
D- Fructose	-
D-Glucose	+
Glycerol	-/+
D-Mannitol	+/-
Lactose	+/-
Sucrose	-
Other tests	
Citrate	-
Indole	-
Gelatin liquefaction	+/-
Gram	variable
catalase	+
oxidase	-
Motility	+

\+ Positive Result - Negative Result

Table.2 Effect of carbon sources on cellulose yield

Sl.No.	Carbon sources	Cellulose yield (g/L)
1	Glucose	0.89 ± 0.20
2	Sucrose	0.43 ± 0.01
3	Lactose	0.66 ± 0.02
4	Maltose	0.47 ± 0.03
5	Mannitol	1.38 ± 0.22

Table.3 Effect of carbon sources on cellulose yield

Sl.No.	Nitrogen Sources	Cellulose Yield (g/L)
1	Peptone	0.86 ± 0.011
2	Urea	0.67 ± 0.03
3	Ammonium sulphate	0.58 ± 0.10
4	Sodium nitrate	0.55 ± 0.02

Table.4 Effect of incubation temperatures on cellulose yield

Sl.No.	Temperature (°C)	Cellulose yield (g/L)
1	25	0.69 ± 0.01
2	30	0.88 ± 0.002
3	35	0.86 ± 0.00
4	40	0.71± 0.02
5	45	0.47 ± 0.01
6	50	0.01± 0.00

Effect of pH on cellulose production

A pH of 7.0 supported the maximum yield of cellulose (0.81 g/L). the results of other pH vaules are given in table-5 and figure-4.

The optimum pH of the culture medium for bacterial cellulose production is in the range of 4.0 to 6.0 the yield of cellulose decreasing below pH=4 (Masaoka *et al.,* 1993). The pH decreases during fermentative production because of the accumulation of gluconic, acetic or lactic acids in the culture broth (Sasithorn Kongruang, 2008).

Esin Poyrazoglu Çoban and Halil Biyik (2011) in their study using *Acetobacter pasteurianus* HBB6 and *Acetobacter lovaniensis* HBB5 strains, found that the highest yield was obtained at pH 6.5 (0.040/0.035 g/L in HS medium, 0.029/0.021 g/L in beet molasses) and the lowest yield was obtained at pH 3.5 (0.007/0.006 g/L) and 4.5 (0.017/0.013 g/L) in HS medium or beet molasses, respectively.

The pH range used by them was 2.5, 3.5, 4.5, 6.5, 7.5 and 8.5. Cell growth and bacterial cellulose yield were not observed in pH 2.5 in their study (Esin Poyrazoglu Çoban and Halil Biyik ,2011).
Studies indicate that the optimal pH range for cellulose production by *A. xylinum* is 4 to 7 (Fontana *et al.,* 1990 and Galas *et al.,* 1990). According to Son et al. (2001), a high level of cellulose production was observed over a broad pH range of between 4.5 and 7.5, and was maximum at pH 6.5 (Son *et al.,* 2003). Pourramezan et al. (2009) studied the effects of various pH (4 to 8) were examined using HS medium. The highest yield was obtained at pH 7 and the lowest yield was obtained at pH 4 (Pourramezan *et al.,* 2009).

Effect of shaking on cellulose production

Shake culture supported maximum cellulose yield (1.12 g/L) as it increased oxygen content of the flask and literatures support that aeration is needed for maximum cellulose production. The results are shown in the table-6 and figure-5.

An economical mass production system of bacterial cellulose (BC) on agitated culture was constructed by Takayasu Tsuchida and Fumihiro Yoshinaga (1997). S.Changjin *et al.,* (2002) showed that shake flask culture were better when compared to the conventional method for the production of Bacterial cellulose (Changjin *et al.*,2002). Tsuchida and Yoshinaga (1997), Kouda et al. (1998), Hwang et al. (1999), Son et al. (2003), Bae et al. (2004) etc., have also shown that shake flask culture supports high quantity of cellulose.

Table.5 Effect of pH on cellulose yield

Sl.No.	pH	Cellulose yield (g/L)
1	5.0	0.56 ± 0.01
2	5.5	0.62 ±0.01
3	6.0	0.68 ±0.02
4	6.5	0.73 ±0.01
5	7.0	0.81±0.01
6	7.5	0.80 ±0.01
7	8.0	0.78 ± 0.03

Table.6 Effect of aeration on cellulose yield

Conditions	Cellulose yield (g/L)
Still culture	0.96 ± 0.02
Shake flask	1.12 ± 0.10

Table.7 Comparison of cellulose production in sugarcane juice media and semi-synthetic media

Culture media	Cellulose yield (g/L)
Sugarcane juice media	1.68 ± 0.01
Semi synthetic media	1.36 ± 0.01

Table.8 Comparison of cellulose production by new and standard strains

Sl.No.	Strain	Cellulose yield (g/L)	
		Sugarcane juice media	Semi-synthetic media
1	*Acetobacter* sp. DR-1	1.68 ± 0.01	1.36 ± 0.01
2	*Acetobacter aceti* ATCC 23747	1.71 ± 0.12	1.42 ± 0.03
3	*Acetobacter xylinum* ATCC 11142	1.65 ± 0.04	1.26 ± 0.12

Table.9 Comparison of cellulose production under optimized and un-optimized conditions

Media	Cellulose yield (g/L)	
	Un-optimized	Optimized
Semi-synthetic media	1.36 ± 0.01	1.82 ± 0.01
Sugarcane juice media	1.68 ± 0.01	2.93 ± 0.01

Table.10 Time profile of cellulose production

Day(s)	Cellulose yield(g/L)
0	0 ±0.00
1	0.09 ± 0.01
2	0.27 ± 0.03
3	0.56 ± 0.01
4	0.98 ± 0.05
5	1.43 ± 0.13
6	1.89 ± 0.02
7	2.01± 0.11
8	2.37 ± 0.17
9	2.91 ± 0.23
10	2.93 ± 0.01
11	2.93 ± 0.20
12	2.93 ± 0.02

Figure.1 Effect of carbon sources on cellulose yield

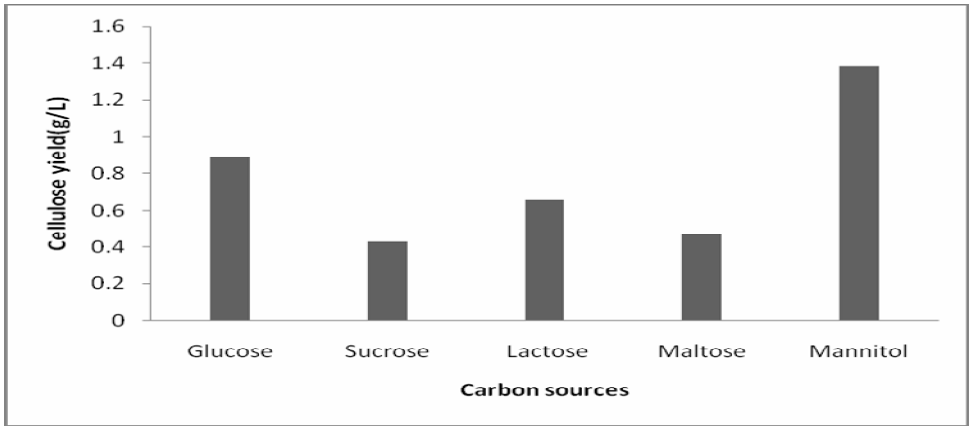


Figure.2 Effect of nitrogen sources on cellulose yield

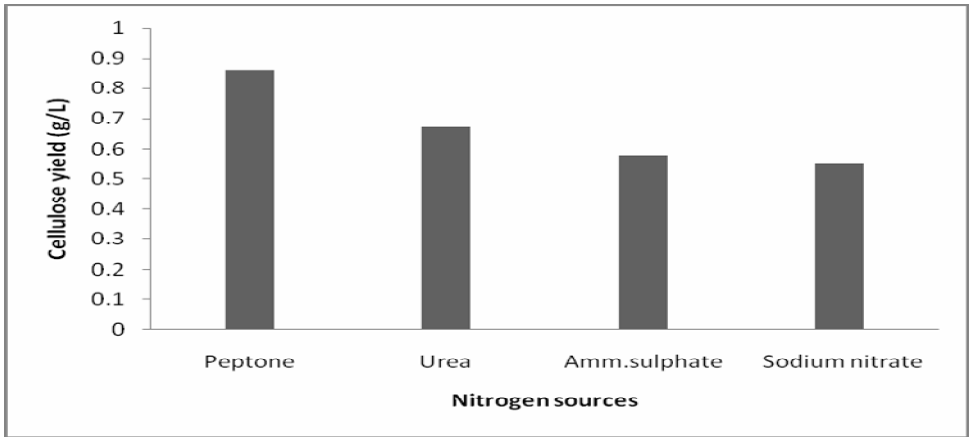


Figure.3 Effect of incubation temperatures on cellulose yield

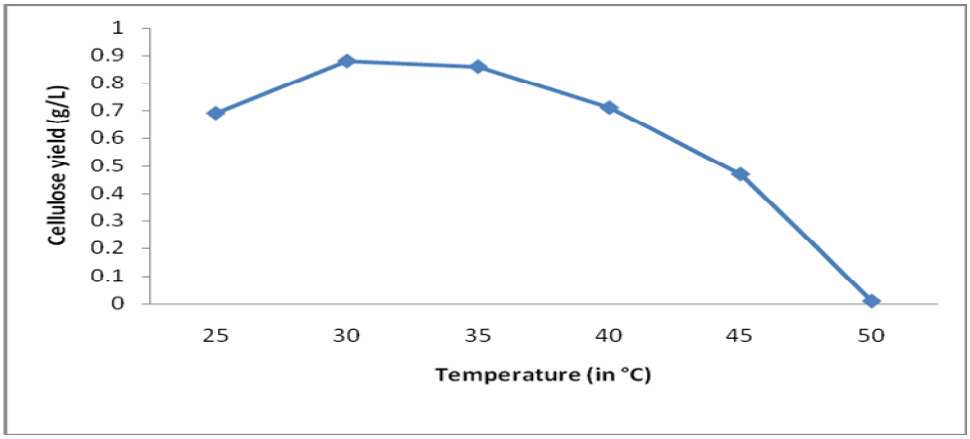


Figure.4 Effect of pH on cellulose yield

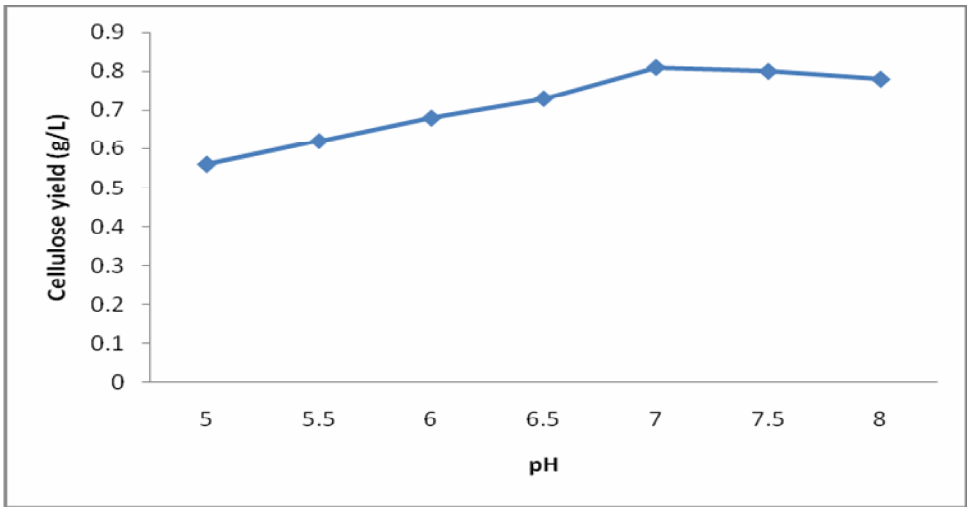


Figure.5 Effect of aeration on cellulose yield

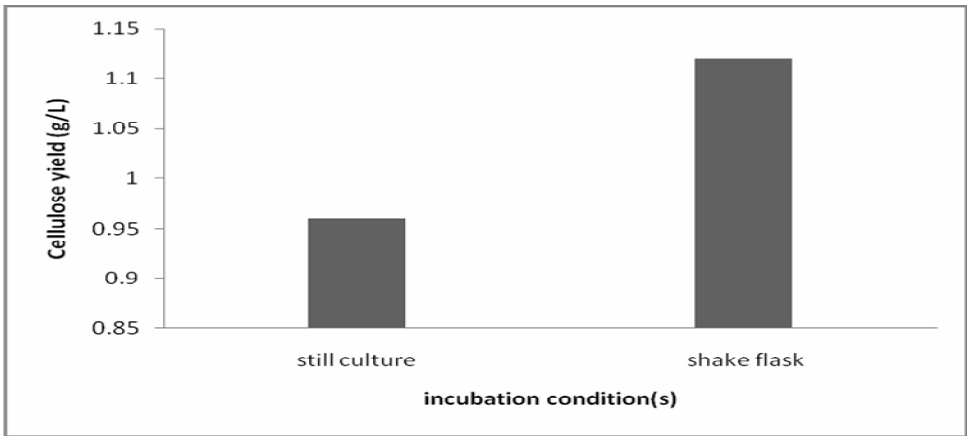


Figure.6 Comparison of cellulose production in sugarcane juice media and semi-synthetic media

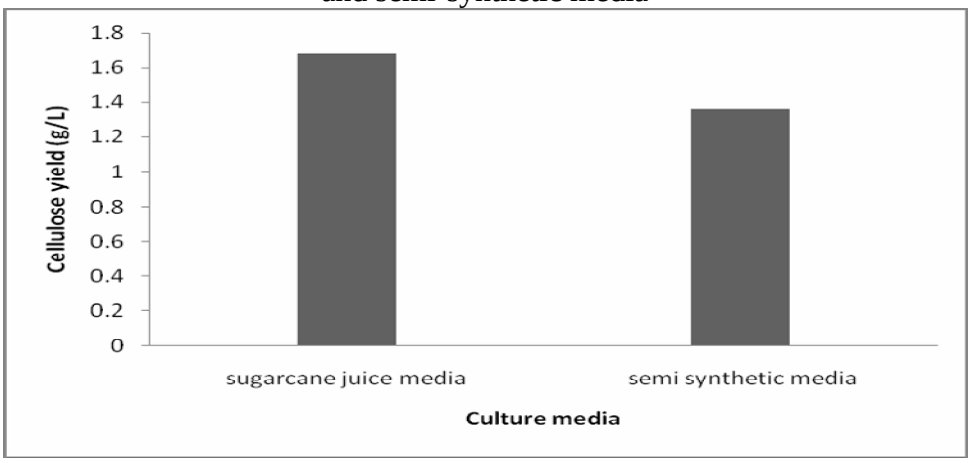


Figure.7 Comparison of cellulose production by new and standard strains

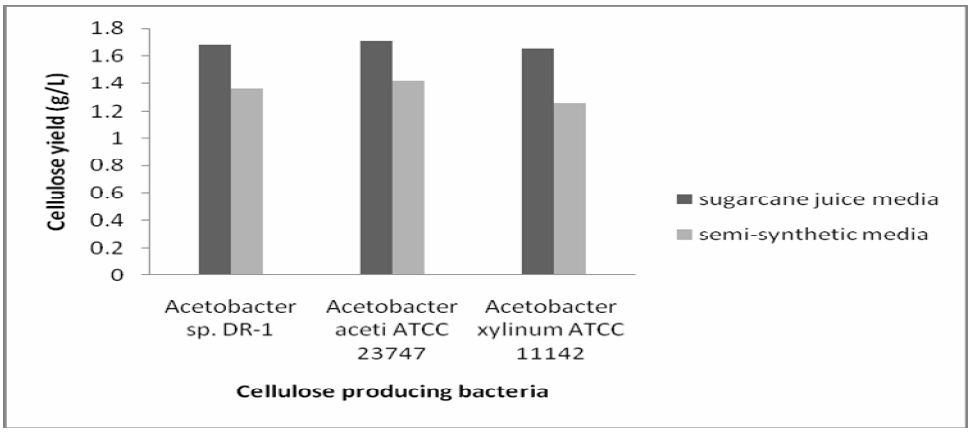


Figure.8 Comparison of cellulose production under optimized and un-optimized conditions

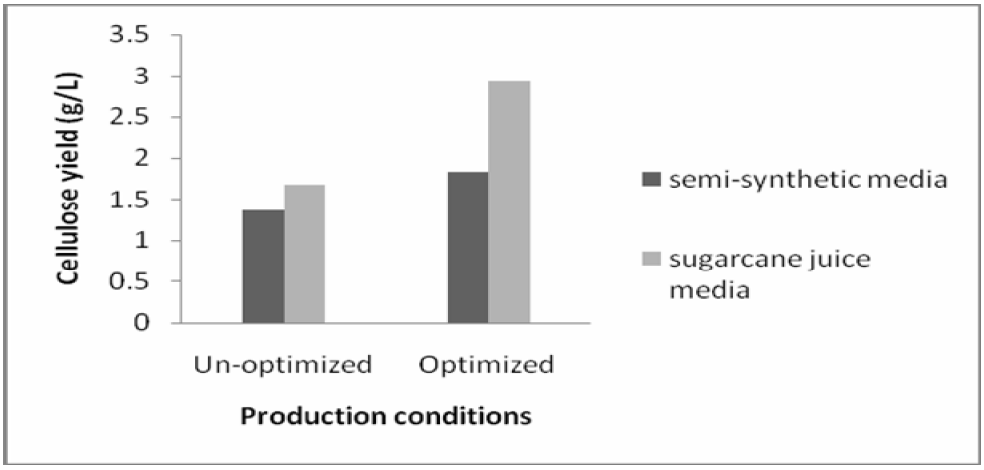
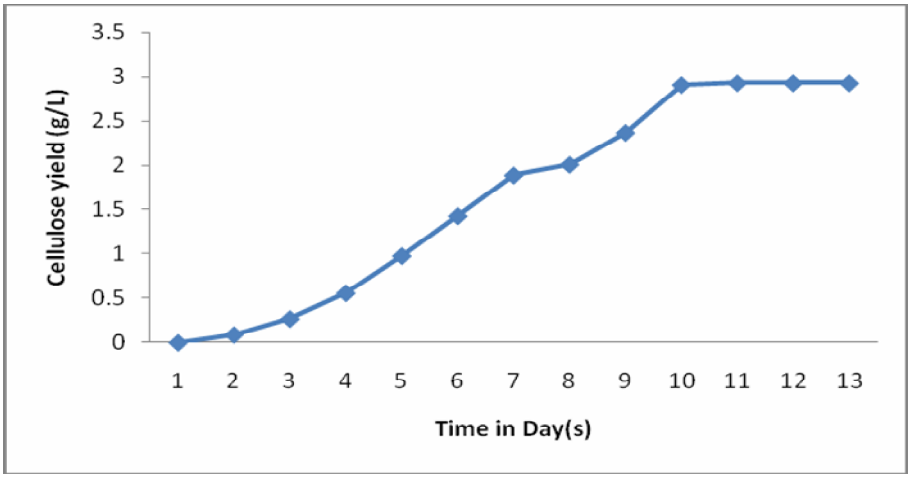


Figure.9 Time profile of cellulose production



The BC yield from the 5 liter stirring bioreactor was the highest (7.94 g/l), followed by that of the shaking flask (5.32 g/l), and the lowest was that of the static tray (4.73 g/l) (Neelobon *et al.*, 2007).

Cellulose production in sugarcane juice

Sugarcane juice supported the highest yield of cellulose when compared to the synthetic media used. There was 1.68 g/L of cellulose produced from sugarcane based media while it was 1.36 g/L from semi synthetic media (Table-7 and Figure-6).

A. xylinum TISTR976 was cultured in coconut juice medium to produce high yield bacterial cellulose (BC) (Neelobon *et al.*, 2007). Biocellulose can be produced using *Acetobacter xylinum*, also growing on broth of sugar cane or tea. The acetic fermentation process is achieved by using the fine sugar as carbohydrate source (Lauro Xavier Filho *et al.*, 2007).

Comparison of cellulose production by the isolated and standard strains

The comparative study shows that the isolated strain yielded maximum quantity of cellulose when compared to the standard strains used in the study (Table-8 and figure-7).

Cellulose production under optimized condition

The cellulose production was carried out under the optimized conditions viz., carbon source, nitrogen source, temperature, pH and shaking. The result was compared with the un-optimized condition. Similarly, the experiment was carried out in sugarcane juice media with the addition of Mannitol and peptone and adjusting the other parameters to the optimum level i.e. temperature at 30°C, pH of 7.0 and a shaking speed of 120 rpm. The results were in favour of optimized conditions. The sugarcane based media gave maximum yield even in the optimized conditions (table-9 and figure-8).

Time profile of cellulose production

The time profile of cellulose production by *Acetobacter* sp. DR-1 in sugarcane juice media and under optimized conditions

shows that the cellulose production starts after 24 hours on incubation and reaches to a maximum by the 10^{th} day, there after there is no increase in the cellulose yield. The results are given in the table-10 and figure-9.

It was proved that the greatest increase in the weight of bacterial cellulose takes place after 7 - 8 days of breeding *Acetobacter xylinum* at a temperature of 30 °C, using a Herstin-Schramm nutrient medium containing: glucose – 2 w/v%, yeast extract – 0.5 w/v%, bacto-pepton – 0.5 w/v%, citric acid – 0.115 w/v%, Na2HPO4 – 0.27 w/v%, MgSO4·7H2O – 0.05 w/v% and w ethanol – 1 v%, added after sterilisation of the base (Surma-Ślusarska *et al.*, 2008).

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