PRODUCTION OF NEW BIO-MATERIAL BY *GLUCONACTOBACTER XYLINUS* USED FOR PROCESSING FOOD PRODUCTS

ABSTRACT:

The present work focused on optimizing the utilization of food processing wastes (such as sugar cane molasses, beet molasses and whey) to produce bacterial cellulose, BC, [(pellicles (using static culture) and/or granules (using agitating culture)] by *Gluconacetobacter xylinus*, suitable for food applications such as sausage wrapping, thickener (for jam and syrup) and binding agent (for sausage, burger, kofta (minced-spiced meatballs) and jam). Schramm-Hestrin medium, containing 2

% sugar, was the starting medium for BC production. Glucose was compared to sugar can molasses, beet molasses and whey as carbon sources in the medium to maximize BC yield, in attempt to form BC production medium with economical profile. The highest cellulose yield was obtained when sugar can molasses was used at a final sugar concentration of 2%. Bacterial cellulose production reached at 1.6g/100ml medium with sugar can molasses, beet molasses produced 1.3g cellulose and whey produced the lowest cellulose yield of 0.3g.

Key words: *Gluconacetobacter xylinus*, *Acetobacter xylinum*, thickener, binding agents, sugar can molasses, beet molasses, whey, bacterial cellulose.

INTRODUCTION:

Cellulose the most abundant natural organic biopolymer exists on earth. It is an insoluble linear polymer consisting of between 2000 - 14000 residues of β-(1⟶4)-D-glucopyranose units. Cellulose is found in two forms, plant cellulose where it is the main structural component of plant cell wall, and bacterial cellulose where it is a primary metabolism product and function as a protective coating. Both, plant and bacterial cellulose, have the same chemical structure, but differ in the physical and chemical properties. Bacterial cellulose, about 1/100 of plant cellulose, is synthesized by several bacterial genera; *Acetobacter, Rhizobium, Agrobacterium and Sarcina.* The most efficient producer is the gram-negative, acetic acid bacteria *Acetobacter xylinum*, which is reclassified as *Gluconacetobacter xylinus* **(Cannon and Anderson 1991, and Steinbüchel, 2004)**. Bacterial cellulose displays distinct advantages over plant cellulose, being absent of lignin or hemicelluloses, completely biodegradable and recyclable, possesses consistent dimensional stability, has a high tensile strength, light weight, has a great water holding capacity, and has a high wet strength **(Klemm *et al.*, 2001 and Sakairi *et al.*, 1998)**. Thus, cellulose can be used in many forms; such as an emulsifier, stabilizer, dispersing agent, thickener, and gelling agent but these are generally subsidiary to its most important use of holding on to water. Although production of bacterial cellulose has been extensively investigated for years, problems have faced the industry especially in its low productivity; therefore some researchers have made many attempts to increase the cellulose productivity from *G. xylinus* using different approaches **(Ishihara *et al.*, 2002 and Ross *et al.*, 1991)**. The present work is an attempt to develop an economic medium and maximize the

U. M. Radwan
Agricultural Sciences Dept., Institute of Environmental Studies and Research, Ain Shams Univ., Abbasiah, Cairo, Egypt.

production of bacterial cellulose from *G. xylinus* using food processing wastes as carbon sources, such as molasses and whey.

MATERIALS AND METHODS:

Microbial strains.

Gluconacetobacter xylinus strain 10245 was obtained from American Type Culture Collection (ATCC, 10801 University Blvd, Manassas, VA 20110).

Schramm and Hestrin medium for activation and subsequent transfer:

Schramm-Hestrin **(Schramm and Hestrin, 1954)** medium used in this study was composed of (w/v): glucose 2%, peptone 0.5% (Difco bacto-peptone), yeast extract 0.5% (Difco), anhydrous disodium phosphate 0.27%, pH was adjusted to 5.0 1N acetic acid. Medium was solidified with Difco Bacto-agar (1.5%). Solid Schramm and Hestrin medium was used for growing colonies on Petri dishes, and then picked and transferred to upgraded medium. Compositions of the upgraded media are shown in Table 1. The initial pH was adjusted to 6.0.

Chemicals.

All chemicals used in this study were purchased from Merck, Germany.

Chemical composition of food processing wastes.

Chemical composition of sugar can molasses, beet molasses and whey, which were used as carbon sources in the production media, were analyzed according to **A.O.A.C. method (1990).**

Production of cellulose.

G. xylinus was inoculated in Schramm-Hestrin media using 2% glucose, fructose, lactose or sucrose as the main source of carbon to choose the best carbon source for BC production. After determining the best carbon source, different concentrations of the selected carbon source were tested for their effect on cellulose production. The best carbon source with its best concentration was compared with three different food processing wastes as carbon source at 2% as the final sugar concentration. Media used for production of cellulose are shown in **Table 2.** Media were prepared in a volume of 300 ml placed in 1L Erlenmeyer flasks. After inoculation, cultures were incubated at 28°C for 7 days either static or agitated. At the end of incubation period, pellicles (static culture) or the granules (agitated culture) of cellulose were collected and washed according to the type of harvest, as described below.

Preparation of cellulose harvest.

Cellulose pellicles were washed with distilled water, 1% NaOH at 90°C for 15min, neutralized with 1% acetic acid and washed with distilled water **(Keshk and Sameshima 2005).** Pellicles were dried in microwave at 150w **(MacCormick *et. al.*, 1993)**, and weighted. In case of cellulose granules, it was recovered by precipitation by mixing of cellulose granules with ethanol at a ration of 1:2, followed by centrifuged at 4500rpm/10mn. Pellets were washed twice with 70% ethanol and dried **(MacCormick *et al.*, 1993).** Yield of BC was calculated as follows:

Cellulose yield (%) = [BC produced (mg) / C source consumed (mg)] X 100

Food processing wastes used for cellulose production.

Different food processing wastes (sugar can molasses, beet molasses and why) were used as a carbon source (at 2% sugar concentration). 2% Glucose was used as a control treatment.

Sugar consumption.

The percentage of sugar consumption during the growth period (7 days) was measured using the method described by **(Keshk and Sameshima 2005)** for glucose, fructose, lactose and sucrose as carbon sources in the media.

BC yield as affect by different glucose concentrations.

Different glucose concentrations were used as a carbon source (0.5-4.0%) in the media, whereas the rest of medium ingredients were fixed. The BC yield was determined as previously described.

The BC production efficiency (%).

The BC production efficiency (%) for: glucose, fructose, lactose and sucrose sugars as a carbon source (at 2% concentration), was calculated as follows:

Production efficiency (%) = Cellulose yield (%) / sugar consumption (%)

RESULTS AND DISCUSSION

Chemical analysis for food processing wastes.

Results, Table(3), shows that highest values of the dry matter and total sugar were found in sugar can molasses followed by beet molasses, and the lowest one was in whey.

Selection of the best carbon source for bacterial cellulose production.

In attempt to determine the best carbon source for cellulose production, Schramm-Hestrin medium was used containing glucose, fructose, lactose or sucrose at 2% final concentration as the main carbon source. Cultures were incubated statically at 28°C for 7 days. As described above. Sugar consumption was estimated from the culture media. Results, Table (4), showed that *G. xylinus* behaved differently toward these types of sugar. Data illustrates that glucose appeared to have the fastest rate of consumption, followed by sucrose, fructose, and the slowest one was lactose. These results are in accordance with the data recorded by **Keshk and Sameshino, 2005**, in which they reported that 97% of glucose was consumed during the incubation period (7days), while less than 50% of fructose was consumed during the same period. Data, Table 5, showed that glucose gave the highest cellulose yield, 40%, followed by fructose with 37.6% yield, then sucrose, 27% and lastly lactose with 8.8%. Results reported by **Takayasu and Fumihiro, 1997**, supporting the current results, showed that *Acetobacter xylinum* can give 20-60% yield of BC, but they assured that 55-60% yield can only be obtained when genetically modified strain is used or when using continuous fermentation and large vessel. Data of production efficiency (%), Table 5, showed that the highest value was 73.7% for fructose, although fructose did not have the highest yield which could be explained by the low consumption rate of fructose, 51.9%, as shown in Table 4. On other hand, cellulose yield and production efficiency for glucose was 40.0% and 41.2%, for sucrose 27.6% and 31.5%, and lactose, the lowest, was 8.8% and 21.9% respectively. The difference in cellulose production between glucose and other sugars can be explained by the high consumption rate of glucose.

Determination of the best carbon source concentration for BC production.

Glucose was used in the cellulose production medium at range of concentrations from 0.5 to 4.0%. Medium was inoculated and incubated as previously described and cellulose yield was measured. Results, **Figure 1,** showed that BC yield increased as the glucose initial concentration in the medium increased from 100 mg/g sugar at 0.5% to 803 mg/g at 2%, after which the BC yield decreased gradually at 2.5% glucose. Therefore, the production peak was achieved at 2% glucose which was the best concentration that produced the highest cellulose yield.

Production of BC in Schramm-Hestrin medium with food processing wastes.

Since 2% showed to be the best carbon concentration to produce the highest cellulose yield, *G. xylinus* was grown in Schramm-Hestrin medium containing sugar can molasses, beet molasses, or whey as the main carbon source in the medium at 2% final sugar concentration. Results showed that 1.6g of BC pellicle/100medium were produced when sugar can molasses was the main carbon source, **Table 6,** beet molasses produced 1.3g, and glucose 1.2g/100ml medium and the lowest figure was in the case of whey which produced 0.3g. Results of related studies found that efficient BC production by *A. xylinum* was achievable in jar fermentors when maintaining lower concentrations of sulfuric acid-heat treated molasses 23-37g/l compared to 48g/l or higher, and this is probably due to the complex composition of molasses **(Bae and Shoda 2005). Vandamme *et al.*, 1998**, were able to improve BC production by *A. xylinum* up to 28 g/l in surface culture, and 9 g/l in submerged culture. Other investigators used *A. xylinum* 23770 and were able to produce 17% more cellulose from low-solid potato effluents than from glucose, indicating that food processing effluents, such as potato, could induce more cellulose production than glucose **(Thompson and Hamilton 2001).**

CONCLUSION

Results showed that using glucose in the cellulose production media gave the highest cellulose yield, followed by fructose, sucrose and lactose. This may be explained by the high consumption rate of glucose. On the other hand, sugar can molasses produced 33% more cellulose than the glucose did as the main source of carbon in Schramm-Hestrin medium by *G. xylinus*.

Table (1): Composition (per 100ml media) of transfer and upgrade media used for *G. xylinus*:

Ingredient / media	Glucose (g)	Molasses (ml)	Peptone (g)	Yeast extract (g)	Na_2HPO_4 (g)	Citric acid (g)
Schramm- Hestrin medium	2.0	0.0	0.5	0.5	0.27	0.15
Schramm-Hestrin plus sugar can molasses (50% sugar)	1.6 1.2 0.8 0.4 0.0	0.8 1.6 2.4 3.2 4.0	0.5	0.5	0.27	0.15
Schramm-Hestrin plus beet molasses (40% sugar)	1.6 1.2 0.8 0.4 0.0	1.0 2.0 3.0 4.0 5.0	0.5	0.5	0.27	0.15

The initial pH was adjusted to 6.0.

Table (2): Composition of the cellulose production media by *G. xylinus*.(per 100 ml media)

Ingredient / media	Glucose (g)	Molasses (ml)	Peptone (g)	Yeast extract (g)	Na_2HPO_4 (g)	Citric acid (g)
Sugar can molasses (50% sugar)	--	4.0	0.5	0.5	0.27	0.15
Beet molasses (40% sugar)	--	5.0	0.5	0.5	0.27	0.15
Whey (5% sugar)	--	40.0	0.5	0.5	0.27	0.15

Table (3): Chemical analysis of food processing wastes used as a carbon source for bacterial cellulose production by *G. xylinus* based on dry weight.

Food processing wastes / component	Sugar can molasses (%)	Beet molasses (%)	Whey (%)
Dry matter	76.0-86.0	75.0-82.0	8.0-10.0
Ash	5.0-7.5	5.0-10.0	5.0-7.0
Total sugar	50.0-52.0	40.0-41.0	4.5-6.0
Reducing sugar	0.5-0.6	0.9-1.0	0.7-1.3
Nitrogen	1.0-3.0	0.2-1.0	5.0-7.0
Calcium	0.01-0.09	0.02-0.12	0.2-0.26
Phosphate	0.03-0.08	0.5-1.6	0.05-0.08
Potassium	2.0-4.0	2.0-6.0	0.1-0.3

Table (4): Sugar consumption (%) by *G. xylinus* during incubation period in Schramm-Hestrin medium with different carbon sources.

Type of sugar	Incubation period (days)						
	1	2	3	4	5	6	7
Glucose	15.0	38.0	74.0	82.0	90.4	95.8	97.0
Fructose	2.0	10.8	25.0	32.6	39.7	44.0	51.9
Lactose	4.7	9.8	18.5	23.7	30.0	35.8	40.2
Sucrose	2.2	9.9	30.2	56.2	72.4	80.1	87.5

Table (5): Yield and production efficiency of bacterial cellulose by *G. xylinus* grown on different carbon sources in the Schramm-Hestrin medium.

Carbon source	Yield (%)[1]	Sugar consumption (%)	Cellulose yield (%)[2]	Production efficiency[3]
Glucose	100.0	97.0	40.0	41.2
Fructose	95.0	51.9	37.6	73.7
Lactose	22.0	40.2	8.8	21.9
Sucrose	69.0	87.5	27.6	31.5

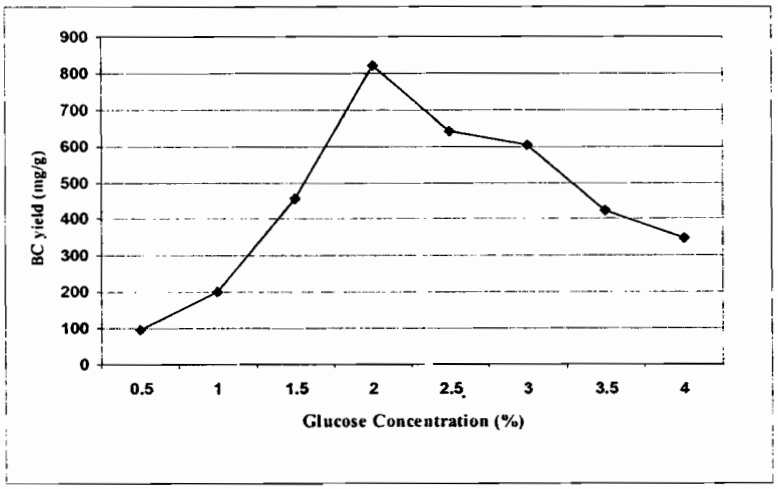
[1]: percentage of BC yield in comparison to that of 2% glucose

[2]: calculated from the dry weight of BC and the weight of carbon source added.

[3]: calculated from the dry weight of BC and the weight of consumed carbon source.

Table (6): Production of BC by *G. xylinus* grown in Schramm-Hestrin medium containing food-processing wastes as carbon source.

Carbon source	BC pellicle yield (g/100medium)
Glucose	**1.2**
Sugar can molasses	**1.6**
Beet molasses	**1.3**
Whey	**0.3**



REFERENCES:

A.O.A.C. (1990). Official methods of analysis (15th edition). Association of Official Analytical Chemists. Washington DC, USA.

Bae, S.O., and M Shoda. (2005). Production of bacterial cellulose by *Acetobacter xylinum* BPR2001 using molasses medium in a jar fermentor. Appl Microbiol Biotechnol. 67(1):45-51

Cannon, R.E. and S. M. Anderson. (1991). Biogenesis of Bacterial Cellulose, Critical Reviews in Microbiology, 17, 435-447

Ishihara, M., M. Matsunaga, N. Hayashi, and V. Tisler. (2002). Utilization of D-xylose as carbon source for production of bacterial cellulose. Enz. And Microbiol. Technol. 31: 986-991.

Keshk, Sh, and K. Sameshima (2005). Evaluation of different carbon sources for bacterial cellulose production. African Journal of Biotechnology. 4(6), pp. 478-482.

Klemm, Dieter, D. Schumann, U. Udhardt and Silvia Marsch. (2001). Bacterial synthesized cellulose - artificial blood vessels for microsurgery. Progress in Polymer Science, Vol.26 (9), pp. 1561-1603

MacCormick, C., J. Harris, A. Gunning, and V. Morris. (1993). Characterization of a variant of the polysaccharide acetan produced by a mutant of *Acetobacter xylinum* strain CR1/4. J. of Applied Bacteriology, 74, 196-199

Ross, O. R. Mayer, and M. Benziman (1991). Cellulose biosynthesis and function in bacteria. Microbiol. Rev. 55: 35-58

Sakairi,N, Sh. Suzuki, K. Ueno, Sang-Mun Han, N. Nishi and S. Tokura (1998) Biosynthesis of hetero-polysaccharides by *Acetobacter xylinum* - Synthesis and characterization of metal-ion adsorptive properties of partially carboxymethylated cellulose. Carbohydrate Polymers. Vol. 37(4), pp. 409-414.

Schramm, M. and Hestrin, S. (1954). Factors affecting Production of Cellulose at the Air/Liquid interface of a culture of *Acetobacter xylinum*, J. Gen, Microbiol., 11, 123-129

Steinbüchel, A. (2004). Biopolymers. Vol. 5, pp 37-46. WILEY-VCH

Takayasu ,T.and Y. Fumihiro (1997). Production of bacterial cellulose by agitation culture systems. Pure & Appl. Chem., Vol.69 (11), pp.2453-2458.

Thompson D.N.; Hamilton M.A. (2001). Production of Bacterial Cellulose from Alternate Feedstocks. Applied Biochemistry and Biotechnology, Vol. 93(1-3), pp. 503-513.

Vandamme, E. J., S. De Baets, A.Vanbaelen, K. Joris, P. De Wulf. (1998). Improved production of bacterial cellulose and its application potential. European Materials Research Society 1997 Spring Meeting, Symposium H, Strasbourg, FRANCE. vol. 59 (1-3), pp. 93-99.

انتاج مادة حيوية جديدة بواسطة بكتيريا *Gluconacetobacter xylinus* واستخدامها فى منتجات الأغذية المصنعة(كمادة رابطة أو مسمكة أو مغلفة)

يتركز البحث الحالى على استخدام مخلفات التصنيع الغذائى (مثل مولاس قصب السكر ومولاس البنجر والشرش) لانتاج سليلوز بكتيرى (على هيئة حبيبات أوطبقة شبيهة بالجلد) صالح للتطبيق فى الصناعات الغذائية مثل تغليف السجق، مادة تثخين (للمربات أو الشربات) أو مادة رابطة (فى تصنيع السجق والبرجر والكفتة والمربى) بواسطة بكتيريا *Gluconacetobacter xylinus* .

استخدمت بيئة Schramm –Hestrin المحتوية على ٢% سكر كبيئة أولية لانتاج السليلوز البكتيرى.

تم مقارنة الجلوكوز مع مولاس قصب السكر ومولاس البنجر والشرش كمصادر رئيسية للكربون بغرض تعظيم محصول السليلوز، فى محاولة لتكوين بيئة اقتصادية لانتاج السليلوز.وقد تم الحصول على أعلى انتاج للسليلوز عندما استخدم مولاس القصب بتركيز ٢% حيث وصل الانتاج إلى معدل ١,٦جم/١٠٠مل بيئة، بينما كان الانتاج فى حالة مولاس البنجر بمعدل ١,٣ جم/ مل بيئة وكان أقل انتاج عند استخدام الشرش قد وصل إلى ٠,٣ جم.