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Edible Bacterial Cellulose Production by *Acetobacter xylinum* 2526 with Response Surface Methodology (RSM) Optimized Processing Parameters in Medium Prepared Using Tofu Whey, Soymilk Okara and Defatted Soy Flour

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A B S T R A C T

A biopolymer bacterial cellulose is obtained by *Acetobacter* species and this biopolymer has unique properties because of that it is considered better than plant-based polymers. In the present investigation attempt has made for low-cost media preparation for *Acetobacter xylinum* NCIM 2526 growth and bacterial cellulose production. Carbohydrate rich fraction was extracted from the soybean industry waste products (Tofu whey, soymilk okara and deffated soy flour) and this fraction was used for the replacement of sugar in the standard medium (*Hestrin schramn*) composition. *Acetobacter xylinum* (NCIM 2526) was cultured in the developed medium and a process is was developed for the maximum yield of bacterial cellulose. The design of the study optimised three conditions: inoculum level 8.47%, incubation temperature 28.85°C and soluble carbohydrate rich fraction 90% (conc.) of soybean by products in dilute form. Yield of bacterial cellulose was 4.66gm/ 100ml of medium which was more than the predicted value 4.55gm/ 100ml of RSM. Finally, it is concluded that the process developed for the production of edible bacterial cellulose increases with use of particular carbon sources in some media and selection of medium and growth conditions greatly affect the yield of edible bacterial cellulose.

Introduction

Bacterial cellulose (BC) is a nano-structured material synthesized by some species of bacteria belonging to the *genera Aerobacter*, *Agrobacterium*, *Rhizobium*, *GluconAcetobacter* (formerly called as *Acetobacter xylinum*), *Acetobacter* (Ross *et al.*, 1991). Bacterial cellulose produced at the air-liquid interface of medium used for the production and it is popularly known as natade-coco. At the air-liquid interface of sugary rich medium is popularly known as nata de-coco. It is an organic high dietary fiber food product, high in cellulose, low in fat and calories and contains no cholesterol.

The cellulose is recognized by the FDA as edible and the *GluconAcetobacter* is a non-pathogenic cellulose-producing food grade bacterium (Kersters *et al.,* 2006). As bacterial cellulose fibrils are highly amorphous and generated as a never-dried membrane in a nearly pure form without lignin and hemicelluloses as that of plant cellulose. Further, more the purity and quality of bacterial cellulose far better as compared to plant based cellulose. BC is characterized by higher purity, higher degree of polymerization, higher crystallinity, higher water-absorbing and holding capacity, higher tensile strength and good biocapatibility, compared to plant cellulose (Klemm *et al.,* 2001; Ul-Islam *et al.,* 2012). These enhanced properties have made BC become considered a kind of highly functional biopolymer which has application potential in bio-medicine, cosmetics, high-end acoustic diaphragms, papermaking, food industry and other areas (Aramwit and Bang 2014; Shah *et al.,* 2013).

In economic terms market potential of thin film bacterial cellulose is in demand including acoustic diaphragms, artificial skin, pulp and paper industry BC is characterized by higher purity, higher degree of polymerization, higher crystallinity, higher water-absorbing and holding capacity, higher tensile strength and good bio-scapatibility, compared to plant cellulose (Klemm *et al.,* 2001; Ul-Islam *et al.,* 2012). Although BC has excellent potential as material in many novel applications, the low yield and high production cost hinder its industrial-scale production and broad range of application. Therefore, looking into inexpensive feedstock as the culture medium is helpful to make BC production more cost-effective (Cavka *et al.,* 2013)

Low-cost waste of soybean industry is rich in sugar, low-molecular weight sugars, glycerol, proteins, vitamins and other nutrients, resulting in high chemical oxygen demand (COD) values. Traditionally, fermentation wastewater is treated by activated sludge. In microbial fermentations, the cost of the fermentation medium can account for almost 30% of the total cost. Medium costs limit commercial use of bacterial cellulose or nata. So use of alternative substrates is one way to reduce the cost of production of bacterial cellulose. Development of a cost-effective culture medium to obtain maximum product yield will solve this problem. Most of the studies on BC production by *Acetobacter* strains have been carried out in media containing pure sugar as carbon source, such as glucose, sucrose, fructose, mannitol, and arabitol (Jung *et al.,* 2010). An alternative sugary nutrient rich substrate that can be used for fermentation is soybean industry waste or low value products. Soybean waste is by product in production of tofu and soymilk. Soybean waste commonly dumps directly to the water sewer and made environmental problems like eutrotification. Soybean waste contains 23% hemicellulose, 16% cellulose dan 28% protein.

This research aimed to formulate the media for production of bacterial cellulose and optimize the processing parameters for production of good quality bacterial cellulose. Furthermore, analysis of BC produced in the formulated media under optimized conditions. Thus, the new media proposed in the present study provides a potentially economical and environmentally-friendly process for the production of BC.

Materials and Methods

Materials collection

Acetobacter xylinum NCIM 2526 was purchased from National Collection of Industrial Microorganisms (NCIM), Pune, India. Hestrin Schramn (HS) medium and other chemicals were purchased from Hi

media. Tofu whey, soymilk okara and deffated soy flour were collected from Centre of Excellence on Soybean Processing & Utilization, ICAR-Central Institute of Agricultural Engineering, Bhopal(India). The laboratory work for the present investigation on the production of cellulose from soybean industry using *Acetobacter xylinum* NCIM 2526 was carried out at Centre of Excellence on Soybean Processing & Utilization.

Formulation of media

The composition analysis of defatted soy slour, soymilk by product (okara) and tofu by product (whey) was done using using standard AACC procedures AACC (2000). Soybean by products: okara, tofy whey and defatted soy flour were analysed for their proximate composition. Soluble carbohydrates or sugars were extracted from the by products with some modification in the method given by Rupérez, 2003. The protocol used is mentioned in the Figure.1.

Culture maintenance and preparation of inoculum

Acetobacter xylinum (NCIM 2526) culture obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. The culture was maintained on HS medium with composition as mentioned by Hestrin and Schramm (Sherif *et al.*, 2006). The composition of medium was (g/100mL): glucose (2g), yeast extract (0.5g), peptone (0.5g), citric acid (0.115g), disodium hydrogen phosphate (0.11g).

The reagents were dissolved in distilled water, the volume was brought up to 100 mL and pH was adjusted to 6.0 with addition of hydrochloric acid or sodium hydroxide and sterilized at 1210C for 15min. *A. xylinum* was streaked on these slants and incubated at 30C for 4 days.

Acetobacter xylinum (NCIM 2526) grown on HS agar slants was inoculated into sterilized media containing glucose (2g), yeast extract (0.5g), peptone (0.5g), citric acid (0.115g), disodium hydrogen phosphate (0.11g). The reagents were dissolved in distilled water, the volume was brought up to 100 mL and pH was adjusted to 6.0 with addition of hydrochloric acid or sodium hydroxide and sterilized at 1210C for 15min. The inoculated media was incubated statically at 30C for 4 days. Sub culturing was done to maintain the purity and viability of microorganism as shown in Figure.2.

Production of bacterial cellulose

Processing conditions for production of bacterial cellulose processing conditions were optimised using response surface methodology. Dependent variables are inoculum level, soluble carbohydrates fraction and incubation temperature. As shown in the table.1.

Harvesting of bacterial cellulose

The Bacterial Cellulose layer formed after 15–20 days was harvested when it was about 0.8–1.0 cm thick, washed repeatedly with water to remove glacial acetic acid. The layer of Bacterial cellulose was immersed in water for 24 h with repeated changing of water to remove the sour odour.

BC purification and quantification

After cultivation, the BC membranes were rinsed three times with deionized water and then soaked in boiling 2% (w/v) NaOH solution for 1 h to remove and dissolve the bacteria cells entrapped in the microfibers.

After turning transparent, the BC membranes were washed with deionized water several times to make the membranes neutralized.

The purified cellulose was dried at 60°C overnight and weighted. For each membrane, triplicate experiments were performed, and the mean values were calculated.

Analysis of bacterial cellulose

Determination of Dry Weight

Cellulose Harvested microbial cellulose washed with NaOH solution 2% (w/v) for 30 min and thoroughly washed with distilled water thoroughly and record the wet cellulose weight and dried at 75°C in an oven for 6 hours, cooled to ambient temperature and record the dry cellulose weight. The dry weight of the cellulose obtained was calculated.

Moisture content

The moisture content (%w/w) of bacterial cellulose was determined based on the weight loss of bacterial cellulose when dried at 75°C.

Moisture content % = (wet weight ─ Dry weight) × 100 Dry weight

Percent yield

Percent yield of BC was calculated by following Equation Percent yield = Dry weight of BC × 100 Weight of carbon source used in production medium.

Statistical analysis

Excel was used for feeding and analysis raw data obtained from different experiments and further Graph pad prism was used to represent data. Data were analyzed by using completely randomized factorial design. Analysis of variance was conducted; when significant effect was detected, the means were separated by Fisher Least Square Analysis. Optimization study data were analyzed by Completely Randomized Design as per the methods described by Steel and Torrie (1980). Storage study data were examined using Factorial CRD. The values for microbial counts were log transformed before analysis.

Results and Discussion

Okara, tofy whey and defatted soy flour used for the extraction of soluble carbohydrates fraction were analysed for their composition as results shown in the table.1

Soluble carbohydrate rich fraction obtained from the soy by products (Okara, tofy whey and defatted soy flour) was used for the formulation of media for *Acetobacter xylinum* (NCIM 2526). In this formulation no additional sucrose was added and this portion of media was replaced with soy by-products soluble carbohydrate rich fraction as shown in the Table.3.

After harvesting, the produced BC in in the standard and developed media was measured for its yield in terms of growth *Acetobacter xylinum* (NCIM 2526) of which refers to the optical density and pH of the per liter of medium as shown in the Table.3.

Optimization of processing conditions for production of high yield of bacterial cellulose using response surface methodology (RSM)

Adequacy of model

The adequacy of model was tested using F-value and coefficient of determination (R^2). The model was generally considered adequate when (a) the calculated F-ratio was more than that of table value; (b) the R^2 value of more than 80 percent (Filmore *et al.*, 1976), and (c) an adequate precision value greater than 4.0.

Table.1 Proximate analysis of soya by-products (Defatted Soy Flour, Soymilk Okara and Tofu whey

Parameters	Defatted Soy Flour	Soymilk by product (Okara)	Tofu by product (whey)
Moisture	4.37 ± 0.03	67 ± 0.61	88.33 ± 0.45
Protein	50.12 ± 0.31	18 ± 0.52	0.81 ±0.29
Fat	-	08 ±0.44	0.78 ±0.32
Ash	6.31 ± 0.19	4.1 ±0.08	3.51 ±0.11
Carbohydrates	43.12 ±0.22	12 ±0.36	0.95 ±0.21

Table.2 Formulation of media with soy by-products extracts and comparison with commercial media

S.No.	Components	Tofu whey –based medium	(SCPS) based media
1.	Sucrose	10%	-
2.	Yeast extract	0.25%	0.25%
3.	K^2HPO^4	0.5%	0.5%
4.	$(NH^4)^2SO^4$	0.6%	0.6%
5.	$MgSO^4$	0.2%	0.2%
6.	Ammonium sulfate	0.25%	0.25%
7.	Calcium sulfate	0.25%	0.25%
8.	Tofu whey	1lit	SCRF with distilled water
pH adjusted to 6.0			

Table.3 Formulation of media with soy by-products extracts and comparison with commercial media

Medium	*2526* (OD)	*2526 (*pH)	*2529* (OD)	*2529*(pH)
HS media	3.666 ± 0.02	4.2±0.04	3.666±0.02	4.2±0.03
Tofu whey –based medium	1.981 ± 0.03	4.7± 0.04	1.882±0.07	5.1±0.04
(SSP) based media	2.771± 0.05	4.6 ±0.03	1.999±0.04	4.9 ±0.08

Table.4 Optimized parameters for bacterial cellulose production and summery of model statics

Optimized parameters					
Cond.	**Value**	**pH**		**Dry wt. of BC (g)**	
Inoculum level (%)	8.47%	Predict	Exp.	Predict	Exp.
Temperature (°C)	28.85°C	3.46793	3.3368	4.55263	4.66
Carbohydrate rich fraction (%)	90%				
Model Summary Statistics					
Quadratic	**pH**	**BBC**			
R-Squared	0.9562	0.9887			
Adj R- Squared	0.9168	0.9785			
Pred R-Squared	0.8583	0.9103			

Fig.1 Extraction of soluble carbohydrates and formulation of media for *Acetobacter* xylinum (NCIM 2526)

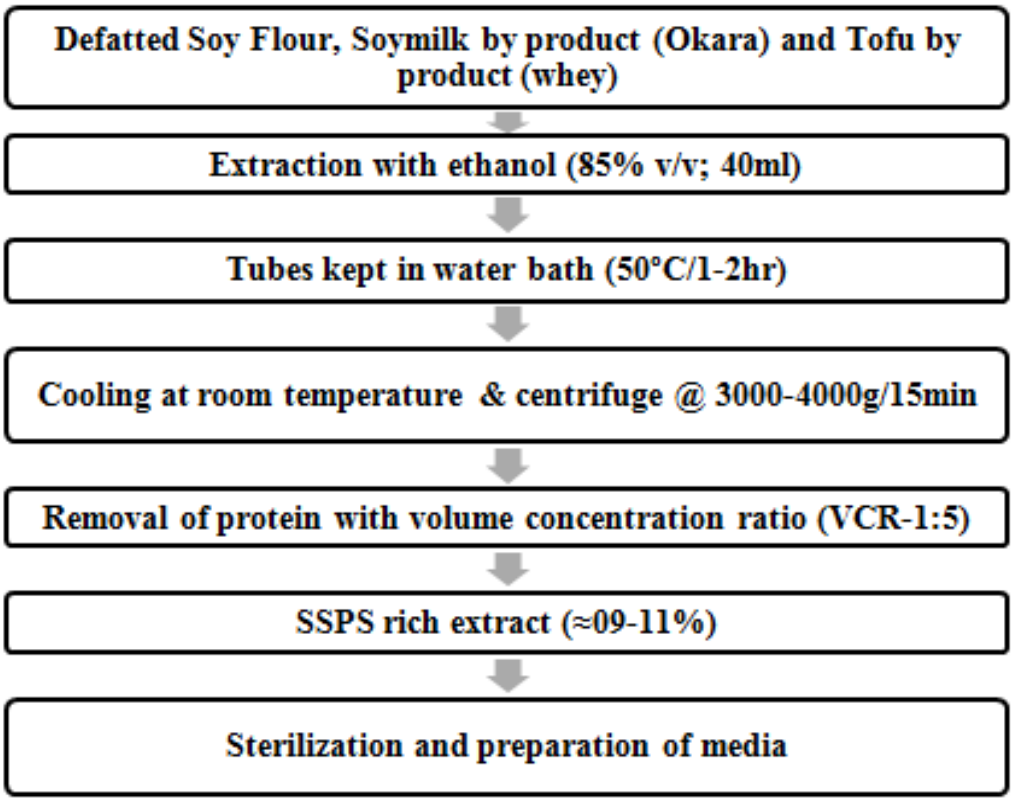
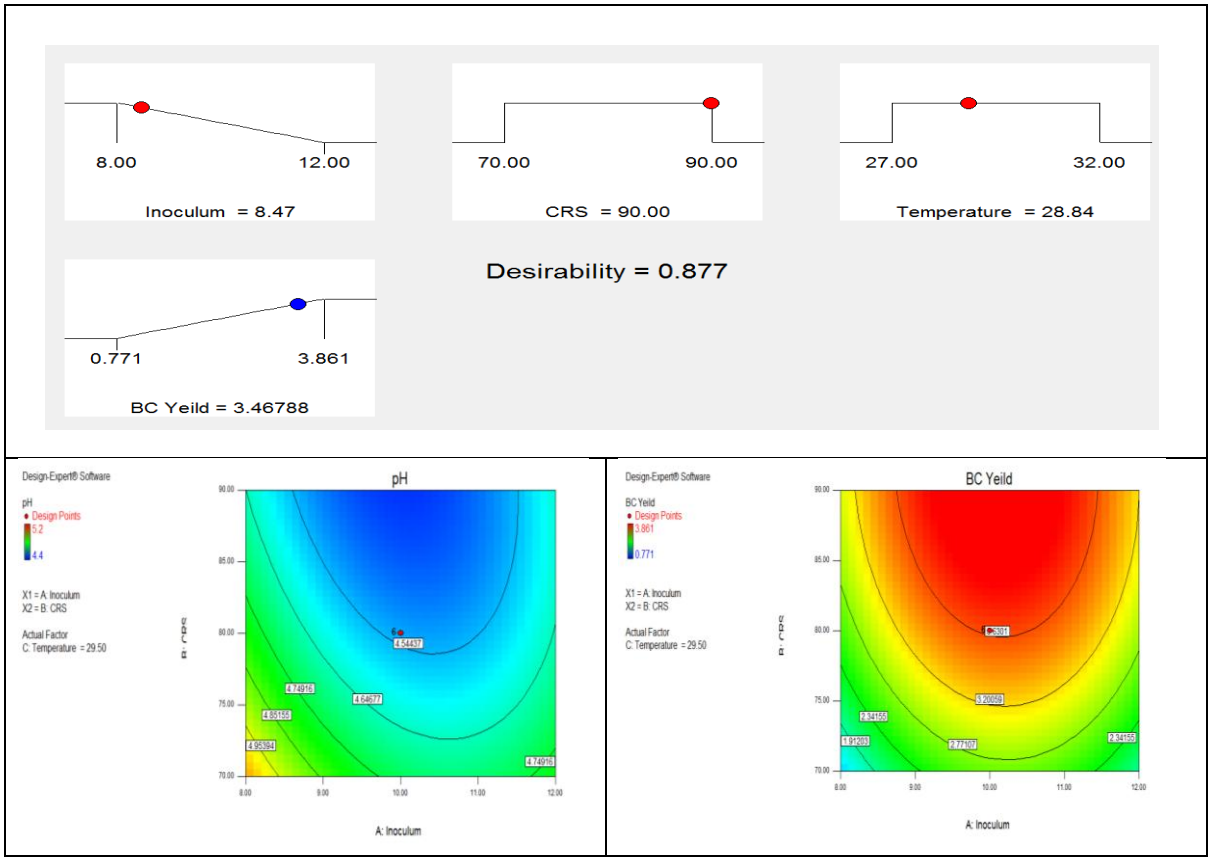


Fig.2

Fig.3 Desirability values and plots obtained in RSM



Response Surface optimization of Variables for production of bacterial cellulose

As there was scanty information on the production of bacterial cellulose by utilising soybean by products, the Central Composite Rotatory Design package (Design Expert 7.0.0) of Response surface methodology technique was applied for the optimizating the levels of various unknown variables e.g. percent of inoculums level, temperature of incubation and percent carbohydrate rich fraction obtained from soy by-products. To optimize the yield of bacterial cellulose in the formulated media and growth *Acetobacter*

xylinum (NCIM 2526). Central composite design of response surface methodology with three levels was employed and results are mentioned in Table.4. A CCRD was used to determine the effects and interactions of three factors.

Highest yield of bacterial cellulose 4.66 gm/ltr of media was obtained at 8.47% inoculum level, 28.85°C, and 90 % Carbohydrate rich fraction (%) with addition of distilled water. Moisture content of the bacterial cellulose obtained with best optimised conditions was 83.27%.

Desirability value is 0.877 the range for all the three parameters in respect of bacterial cellulose yield and plots obtained in application of RSM are shown in the Figure 3. The results indicate the optimized process for the production bacterial cellulose by utilization of soy industry by products is a potential and economic approach.

Future Prospects

Due to ever increasing population in urbans, the management of food industry waste is a major challenge. Effective recycling of this waste at source is one of the most efficient waste managements. Thus, using this technology, soybean industry by products or waste can be recycled at source for minimizing environmental pollution with additional economic benefit and employment generation.

Bacterial cellulose was produced by *Acetobacter xylinum* (NCIM 2526) using Okara, tofy whey and defatted soy flour as substrates proved to be an efficient substrate for production of bacterial cellulose when compared with standard medium or substrate. In addition, it does not involve toxic and any kind of hazardous materials in producing Bacterial cellulose, which is excellent and suitable for safe environments such as food and medical applications. Cellulose production increases with use of particular carbon sources in some media, but not in others and yield is greatly affected by selection of media. It could be concluded that optimized conditions are suitable for bacterial cellulose production.

References

Approved laboratory methods. St. Paul: American Association of Cereal Chemists; 2000.

Aramwit P, Bang N. The characteristics of bacterial nanocellulose gel releasing silk sericin for facial treatment. BMC Biotechnol. 2014 [cited 2017 May 04];14:1. DOI:10.1186/s12896-014-0104-x.

Cavka A, Guo X, Tang S-J, *et al.*, Production of bacterial cellulose and enzyme from waste fiber sludge. Biotechnol Biofuels. 2013 [cited 2017 May 04];6:1. DOI:10.1186/1754- 6834-6-25

Filmore, E. B., Kramer, A. and Gerald, K., (1976). Response surface techniques. *In Filmore FE, Kramer A, Gerald K (eds.), Systems Analysis for Food Industry AVI, Westport, Connecticut*, 127.

Jung, H. I., Lee, O. M., Jeong, J. H., Jeon, Y. D., Park, K., Kim, H. S., An, W. G. and Son, H.J., Production and characterization of cellulose by *Acetobacter* sp. V6 using a cost effective molasses-corn steep liquor medium. Appl. Biochem. Biotechnol., 2010; 162: 486-97.

Kersters, K., Lisdiyanti, P., Komagata, K. and Swings, J., The family *Acetobacter*aceae: the genera *Acetobacter*, Acidomonas, Asaia, *GluconAcetobacter*, Gluconobacter and Kozakia. (in) The Prokaryotes.

Springer, New York. 2006; 163-200.

Klemm D, Schumann D, Udhardt U, *al.*, Bacterial synthesized cellulose-artificial blood vessels for microsurgery. Prog Polym Sci. 2001; 26:1561–1603.

Ross P, Mayer R, Benziman M. Cellulose biosynthesis and function in bacteria. Microbiol Rev. 1991; 55:35–58.

Rupérez P, Toledano G: Celery by-products as a source of mannitol. Eur Food Res Technol 2003, 216:224-226.

Shah N, Ul-Islam M, Khattak WA, *al.*, Overview of bacterial cellulose composites: a multipurpose advanced material. CarbohydrPolym. 2013; 98:1585–1598.

Sherif K., Taha R. and Sameshima K. (2006), "Bacterial Cellulose Production from Beet Molasses", African Journal of Biotechnology, 5 (17): 1519-1523.

Steel, R. G. D., and Torrie, J. H., (1980)." Principles and procedure of statistics - a biometrical approach". 2nd Ed. McGraw-Hill Kogakusha Ltd., Japan.pp:137.

Ul-Islam M, Khan T, Park J K. Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. Carbohydr.Polym. 2012;88:596–603.

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