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Drying Characteristics of Bacterial Cellulose Produced From Fermentation of Black Tea by Symbiotic Colony of Yeast and Bacteria

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Abstract: ***Bacterial cellulose (BC) is one of the important biomaterial which has touched almost all emerging technological fields. Industries produce BC by fermentation process using Gluconoacetobacter xyllinum, which cellulose turns out to be expensive and short period of shelf life. Considering these difficulties faced in the present scenario, this experiment was designed to produce bacterial cellulose from fermented tea or Kombucha tea by Symbiotic colony of bacterial and yeast (SCOBY) and drying characteristic were optimized for longer shelf life, also determining their equilibrium moisture content and critical moisture content.***

Keywords: Bacterial cellulose, Kombucha, SCOBY, Equillibrium moisture content, Critical moisture content

1. Introduction

Bacterial cellulose is an extracellular compound produced during the symbiosis of bacteria and yeast, the bacteria strain is *Gluconoacetobacter Xylinum* and the yeast strain *Saccharomyces Cervicae*, together these strains undergoes symbiosis utilizing the nutrients in the tea broth, they produce microfibrils which forms covalent bond among themselves and creates a bacterial cellulosic (BC) pellicle or mat, floating on the surface of the broth. [1]

The synthesis of bacterial cellulose is a multistep process that involves two main mechanisms: the synthesis of uridine diphosphoglucose (UDPGIc), followed by the polymerization of glucose into long and unbranched chains (the β-1→4 glucan chain). Specifics on the cellulose synthesis have been extensively documented. The former mechanism is well known while the latter still needs exploring. The production of UDPGIc starts with carbon compounds (such as hexoses, glycerol, dihydroxyacetone, pyruvate, and dicarboxylic acids) entering the Krebs cycle, gluconeogenesis, or the pentose phosphate cycle depending on what carbon source is available. It then goes through phosphorylation along with catalysis, followed by isomerization of the intermediate, and a process known as UDPGIc pyrophosphorylase to convert the compounds into UDPGIc, a precursor to the production of cellulose. The polymerization of glucose into the β-1→4 glucan chain has been hypothesized to either involve a lipid intermediate or not to involve a lipid intermediate, *A. xylinum* usually converts carbon compounds into cellulose with around 50% efficiency. [2]

Downstream process involves isolation of BC pellicle by acid-alkali treatment, and drying unit operation is conducted in order to remove the excess moisture from the fabric which inhibits BC from adhering with applicative substances such as Ag^+ Nano-particles, antibodies, drug or protein based molecules. BC pellicles are extremely hydrophilic, they tend to absorb water readily resulting in swelling, which is due to osmotic action; the solvent molecules penetrate across the cellulosic layer and occupy the intermolecular voids. [3] Drying action removes the water from the surface of the BC pellicle thereby the imbibing water from the intermolecular voids is removed when it reaches the surface of the pellicle. Equilibrium moisture content and critical moisture content can be determined while conducting the drying operation using a Tray dryer under variable temperature. Equilibrium moisture content can be defined as the amount of moisture content after which there is a rapid diffusion of moisture as per driving force Critical moisture content can be defined as the amount of moisture content after which rate of moisture removal is almost 0. [4]

2. Literature Survey

Study so far conducted was purely on the interest of effective production of bacterial cellulose, its quantification, and applied molecular biological techniques on *A. xyllinum* to increase the yield of production. [5] But very less study has been conducted on bacterial cellulose produced from kombucha tea, industrial investors are facing difficulty in accepting the BC produced form Kombucha, due to their hygroscopic nature, odor and colour retention. This study was conducted to solve those issues faced on quantifying the shelf-life of BC from kombucha tea, so that it can be accepted by the investors, as an alternative cellulose source other than nitrocellulose.

3. Materials and Methodology

3.1. SCOBY Mother Culture

SCOBY mother culture was obtained from kombucha tea Synergy. For preparation of the tea media Brooke Bond Taj Mahal dip tea bags were used, each weighing 2 g. About 500 ml of black tea was prepared with sucrose concentration of 60 g/l, and incubated for 15 days. After fermentation the SCOBY sub-culture with 6mm thickness floats on the surface of the media which was used for further process. [6]

3.2 Fermentation of Black Tea Broth

The sub-culture obtained from the mother culture was transferred into the black tea media and kept for incubation, under static condition, at room temperature (30 ^{o}C), sucrose concentration 60 g/l and at pH 4.5. [7]

3.3. Chemical Treatment

The BC pellicle was removed from the media and treated with warm distilled water to detach the SCOBY (the yeast and bacterial strains) from the BC, then 50 ml of 5% 0.5 N NaOH was used to clean the presence of cellular debris, followed by 1% 0.5 N Acetic acid to remove the traces of microbial cells and debris and finally with cold distilled water to remove the chemicals added for its treatment. [8]

3.4. Drying Unit Operation

Initial weight of the sample quantity was observed, i.e. pre dried BC pellicle after its treatment, and it was soaked into cold distilled water for 1 h. The wet weight of the BC pellicle was observed to be same as that of the weight of the BC pellicle after chemical treatment, the wet BC pellicle was subjected into the tray dryer (Dipthi Engineering). Before introducing the wet BC pellicle the blower and heating coil was turned on, until constant temperature was attained, the fabric was kept on the drying pan of the dryer time interval was noted for every 0.5 g of moisture removed and tabulate the readings, until the initial weight is attained. [9][10]

Figure 1: Tray Dryer used for the unit operation on the bacterial cellulose

$$\text{Moisture content (X)} = \frac{\text{(Initial moisture – Moisture removed)}}{\text{Weight of the dry sample}}$$

$dX = X_1 - X_2$

$dT = T_2 - T_1$

Rate of drying N (g of moisture removed h-1 m-2) =

$$\frac{Ls}{A} \times \frac{dX}{dT} \times 10^{-3}$$

L_S = Weight of the dry BC sample, g = 1.000 g

dX = g of moisture removed per g of dry solid

dT = Time taken to remove moisture, sec

4. Results and Discussion

The BC pellicle is extremely hydrophilic, and prone to moisture, so drying characteristics plays an important role in determining how efficiently moisture can be removed from the wet BC pellicle, using a tray dryer. From table 1, the suffix 1-8 resembles for variable feed temperature 40 ^{o}C, 50 ^{o}C, 60 ^{o}C, 70 ^{o}C, 80 ^{o}C, 90 ^{o}C, 100 ^{o}C and 110 ^{o}C. From figure 2, the Equilibrium moisture content was found to be 3g and Critical moisture content was obtained 1 g. [10]

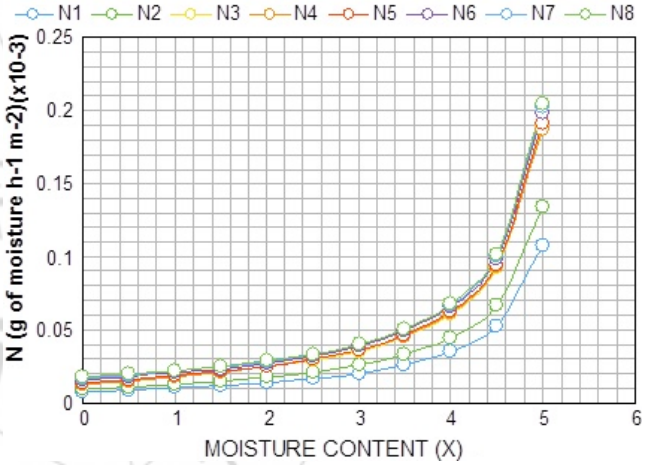

Figure 2: The curve represents the equilibrium moisture content and critical moisture content, the plot is between Drying rate and moisture content for variable temperatures

Table 1: Represents the drying rate (N) and moisture content (X) obtained during experimentation with BC sample.

Sl No.	X	N1	N2	N3	N4	N5	N6	N7	N8
		g of moisture h-1 m-2 ($X10^{-3}$)							
1	5	0.10771	0.13411	0.18898	0.18728	0.19071	0.19798	0.20182	0.2038
2	4.5	0.05317	0.06663	0.09239	0.09322	0.09492	0.09899	0.10091	0.1019
3	4	0.03511	0.04423	0.06096	0.06168	0.06299	0.06599	0.06727	0.06793
4	3.5	0.02615	0.03305	0.04529	0.04609	0.0463	0.04949	0.05046	0.05095
5	3	0.02077	0.02631	0.03584	0.03653	0.0366	0.0396	0.04036	0.04076
6	2.5	0.01717	0.02126	0.02961	0.03017	0.03026	0.03253	0.03364	0.03397
7	2	0.01448	0.0178	0.02511	0.0256	0.02579	0.02761	0.02883	0.02911
8	1.5	0.0125	0.01527	0.0213	0.02172	0.02238	0.02378	0.02499	0.02547
9	1	0.01096	0.01323	0.0179	0.01835	0.01937	0.02089	0.02202	0.02264
10	0.5	0.00961	0.01159	0.0152	0.01554	0.01647	0.01854	0.01954	0.02022
11	0	0.00841	0.01001	0.01274	0.01303	0.01403	0.01658	0.0175	0.01817

In the beginning of the operation sample was at colder temperature than its ultimate temperature, as the temperature increases the rate of evaporation increases. Considerably, if the sample is initially at higher temperature then the rate of evaporation will show an effective result. The figure 3 represents the relation between time taken and moisture content, where it is clear at higher temperature moisture is effectively removed at 60 ^{o}C an effective removal of moisture is attained with time, after which there is less deflection in time. For best, drying results samples can be initially kept at 90 ^{o}C and change it to 60 ^{o}C near to equilibrium moisture content. This may result an effective drying in less expense of time. [10]

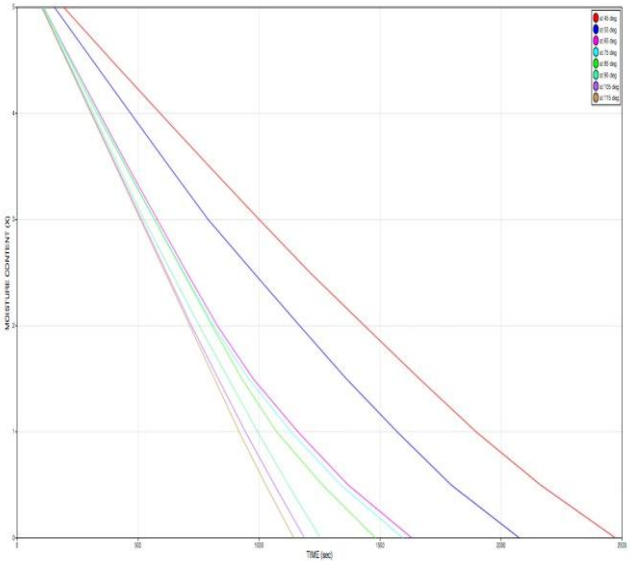

Figure 3: The curve represents the relation between moisture content and time required for drying, each segments represents variable temperatures.

Table 2: Represents the relation between Moisture content and time required for drying for variable temperatures, with their values on experimentation with BC sample.

X	t1	t2	t3	t4	t5	t6	t7	t8
Moisture content	(sec)							
5	193	155	110	111	109	105	103	102
4.5	391	312	225	223	219	210	206	204
4	592	470	341	337	330	315	309	306
3.5	795	629	459	451	449	420	412	408
3	1001	790	580	569	568	525	515	510
2.5	1211	978	702	689	687	639	618	612
2	1436	1168	828	812	806	753	721	714
1.5	1663	1361	976	957	929	874	832	816
1	1897	1571	1161	1133	1073	995	944	918
0.5	2163	1794	1368	1338	1262	1121	1064	1028
0	2473	2076	1632	1595	1482	1254	1188	1144

5. Conclusion

Bacterial cellulose is of commercial interest for many of the same reasons that cotton fields and forests attract the industrialist's attention. It is likely that the maximum capacity of bacteria such as *A. xylinum* present in SCOBY to produce cellulose has not reached full expression, although reports on industrial production do indicate an ultimate potential that is both greater and more obtainable than expected.

Several studies have shown effective cellulose yield, the cellulose produced were productive in medical, and industrial market. However, the issue regarding dried bacterial pellicle was not effectively solved, where dried bacterial cellulose shows longer shelf life compared to that of cultured and treated once. The effective moisture content and critical moisture content was obtained for various feed temperature and were optimized.

6. Future Scope

The future prospect can be considered as the development of bacterial cellulose producing industries to utilize bacterial cellulose from kombucha tea and increase its shelf life by drying them. There is a possibility that these dried BC pellicle can be stored and marketed to industries demanding them, with almost the same effectiveness as that of commercial bacterial cellulose.

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Arpan Kumar Basak is currently perusing his 4th semester M.Tech, in biotechnology from National Institute of Technology, Durgapur. He has graduated in Biotechnology engineering from Visveswarya Technological University, Belgaum. Having a diploma degree on Fermentation technology and Bioprocessing, makes him technologically skillful in matters of industrial biotechnology. In addition to all these he has specialized in HPLC and Bio-instrumentation. He has participated in industrial projects developed in CSIR-CMERI, Durgapur and Galaxy Biotech Pvt. Ltd, Kolkata.