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Absorption of anthocyanin dye and its first order kinetics on bacterial cellulose produced by fermentation of black tea

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Abstract: *Bacterial cellulose (BC) is one of the most important biomaterial in food and nutraceutical industries, these industries utilize BCto immobilize anti-oxidants and flavonoids inside, as they are easily digestible. Industries produce BC by fermentation process using Gluconoacetobacterxyllinum, which cellulose turns out to be expensive. This experiment was designed to produce bacterial cellulose from fermented tea or Kombucha tea by Symbiotic colony of bacterial and yeast (SCOBY) and absorption of anthocyanin dye on BC pellicle was studied, dye retention capacity were obtained for samples kept in various dye loading% and soaked for different time periods, dye retention capacity optimized the time period and dye loading for BC.*

Keywords: *Absorption, Anthocyanin,Bacterial cellulose, Kombucha, SCOBY*

I. Introduction

Bacterial cellulose is an extracellular compound produced during the fermentation of tea by symbiosis of bacteria and yeast, the bacteria strain is GluconoacetobacterXylinumand 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 UDPGIcpyrophosphorylase 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% capacity.[2][3]

Anthocyanin arecoloredpigmentsandthesearefoundprofuselyinplantkingdom.Thecolorsimpartedbythese pigmentsareblue,redandpurple.Inflower,fruit,stem,leavesandrootofplants.Theysolubleinwaterand generallyoccurintheaqueouscellsap. Anthocyanin can be used in colourization of the BC pellicle. If BC pellicle is used as vegetable leather or bio-couture.[4]

Absorption of anthocyanin not only depicts the colourization of the BC pellicle but it also derives a quantitative approach of solvent retention capacity of the BC pellicle. Solvent retention capacity is the amount of solvent molecules retained by the material on which the solvent was subjected, it can also be defined as the ratio of concentration of solvent to the concentration of solvent in which the material was soaked into. Similarly, Dye retention capacity can be determined to estimate the amount of dye the material has retained.This process can be applicatedin storing various antioxidants such as cumin, turmeric, lycopene etc. This can be beneficial for food and food supplement industries. A convenient source of bacterial cellulose along with immobilized antioxidants.[5]

II. Materials and methodology

2.1. 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]

2.2 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]

2.3. 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]

2.4. Action of dye on bio-fabric

Dry BC pieces (5mm x 5mm x 0.5mm) were incubated (5mm x 5mm) for variable time period, in a solution of anthocyanin and ethanoic acid (5:1), agitated on aspynx agitator. After incubation, pieces of BC sample were kept for drying in a hot air oven, for 2 h at 80^{o}C. The dried samples are placed inside the test tubes containing ethanoic acid or ethanoic HCl and kept for 1 h, the dye from the sample starts retaining, imparting the amount of dye it has absorbed during incubation, the dye settled at the bottom of the tube was agitated by the spynx rotor and absorbance under 520nm was taken in spectrophotometer, series of tests were conducted for several dye loading concentrations such as 10%, 30%, 50%, 70%, and 90% of the dye having a concentration of 1520 µg/ml, and the absorbance was taken into consideration according to the concentration of dye, was estimated from the standard anthocyanin curve and the concentration of anthocyanin absorbed was estimated, and dye retention capacity was calculated referred in Equation 1. For different dye loading, a relation was estimated between dye retention capacity and time of incubation (30, 60, 120, 180, 720, and 1440 mins). A first order kinetics was determined for different dye loading concentration referred in equation 2 and 3 along with determination of k value for different dye loads equation 4. [9]

(1) Dye retention capacity % = $\frac{\text{Conc .of dye measued after incubation}}{\text{Conc .of dye loaded}} \times 100$

The general equation for 1st order kinetics,

(2) $\frac{d[D]}{dt} = -k[D]^1$

Where,

kis the specific rate constant

The first order differential equation integrates to

(3) $\ln[D] = \ln[D]_0 - k \cdot t$

(4) $k = -\frac{\ln[D] - \ln[D]_0}{t}$

Thus, the logarithm of the concentration versus time can be linear or non-linear function of the time with $[D]_o$equal to the initial concentration at time zero and slope value is k.If non-linear then a graph of 1/[D] vs time is considered for a second order kinetics.

III. Results and discussions

3.1. Dye retention capacity

BC pellicle after drying, provides commercial bio-fabric, this BC pellicle has the capability of absorbing solvent molecules, BC pellicle when subjected in anthocyanin solution,and it readily takes up the dye upon incubation set up for various dye loading and time period referred in Table 1. Dye retention capacity was determined and observed maximum on 50% loading for 180 mins. From the graphical representation, it was observed that for every incubation period there's an increase in dye retention capacity. After 180 mins the dye retention capacity reaches a saturation point. Similarly after 50% loading the dye retention capacity reaches a saturation point. The images represents the uptake of dye at saturation point for experimental samples and a whole BC pellicle. Fig. 3 represents the relationship between dye retention capacity and time for variable dye loads. [9]

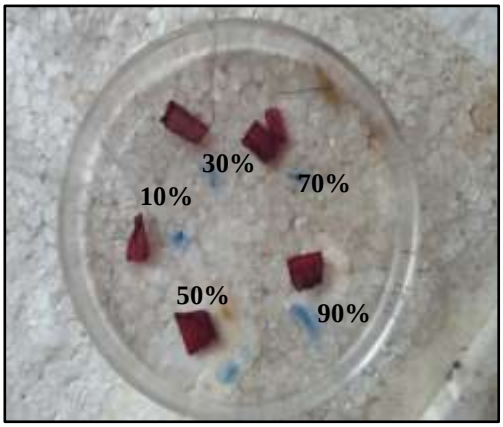

Figure 1: BC fragments at different dye loading.

Figure 2:BC pellicle soaked at optimum dye condition, image taken after drying after drying.

Table 1: Represents the dye retention capacity, obtained for various dye loading% for various time period.

Dye Loading%	10	30	50	70	90
Time (mins)	Dye retention capacity				
0	0	0	0	0	0
30	1.118421	5.701754	5.394737	3.242481	2.55848
60	3.026316	13.26754	19.27632	28.24248	26.38889
120	3.388158	38.92544	46.31579	40.71429	41.70322
180	30.26316	79.82456	83.88158	73.26128	72.62427
720	10.92105	77.96053	83.55263	71.38158	70.86988
1440	9.901316	77.96053	83.42105	71.75752	70.94298

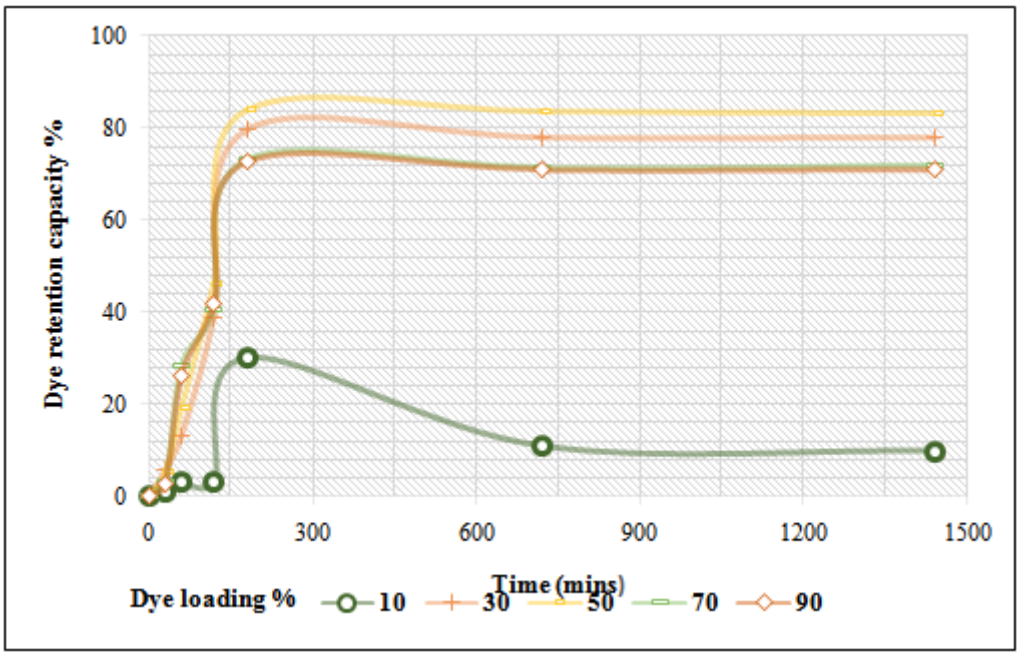

Figure 3: The curve represents the relation between dye retention capacity of BC samples for various dye loading.

3.2. Dye absorption kinetics

A first order kinetics was derived for samples with different loading where a plot of -Ln [D] versus time was analyzed Fig. 4. The analysis states that there is a gradual increase in the concentration of the substrate intake by the BC pellicle with time which reaches a point of saturation after 180 mins. The rate constant was estimated for each dye loading, and a graph was plot against rate constant k verses time. [10]

Table 2: The table represents the concentrations of dye liberated out form the BC sample, which was taken up during incubation with anthocyanin dye and ln[D] for 1st order kinetics from equation 3.

Dye Loading %	**10**		**30**		**50**		**70**		**90**	
Time (mins)	**Dye conc. [D] µg/ml**	**- ln [D]**	**Dye conc. [D] µg/ml**	**- ln [D]**	**Dye conc. [D] µg/ml**	**- ln [D]**	**Dye conc. [D] µg/ml**	**- ln [D]**	**Dye conc. [D] µg/ml**	**- ln [D]**
0	0		0		0		0		0	
30	1.7	0.530628	26	1.740774	41	3.713572	34.5	3.540959	35	3.555348
60	4.6	1.526056	60.5	2.585321	146.5	4.987025	300.5	5.705448	361	5.888878
120	5.15	1.638997	177.5	3.661648	352	5.863631	433.2	6.0712	570.5	6.346513
180	46	3.828641	364	4.379831	637.5	6.457554	779.5	6.658653	993.5	6.901234
720	16.6	2.809403	355.5	4.356203	635	6.453625	759.5	6.63266	969.5	6.87678
1440	15.05	2.711378	355.5	4.356203	634	6.452049	763.5	6.637913	970.5	6.877811

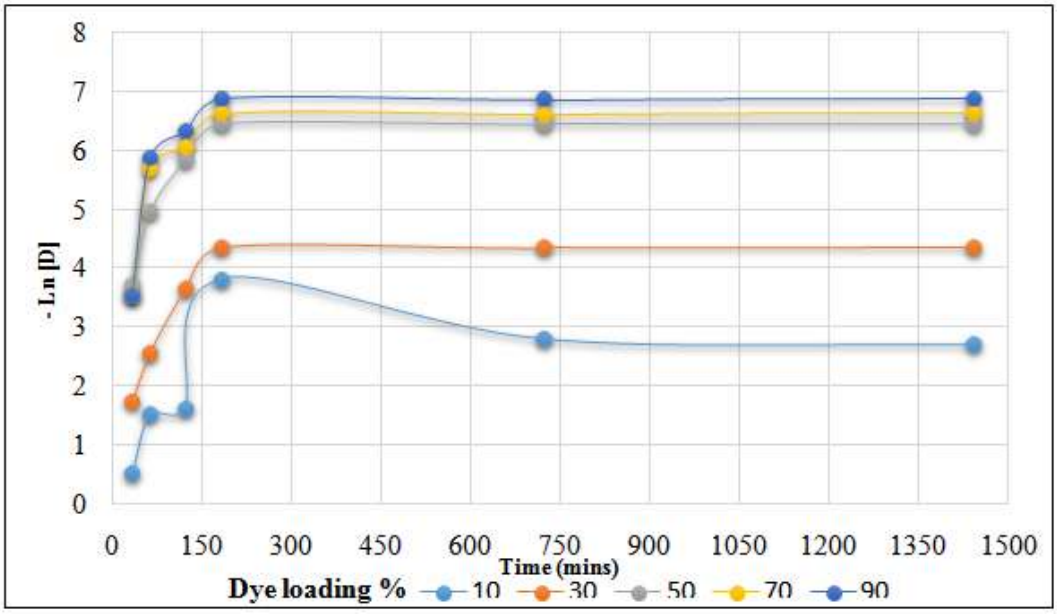

Figure 4: The curve shows the nonlinear nature of the 1st order kinetics for various dye loading%.

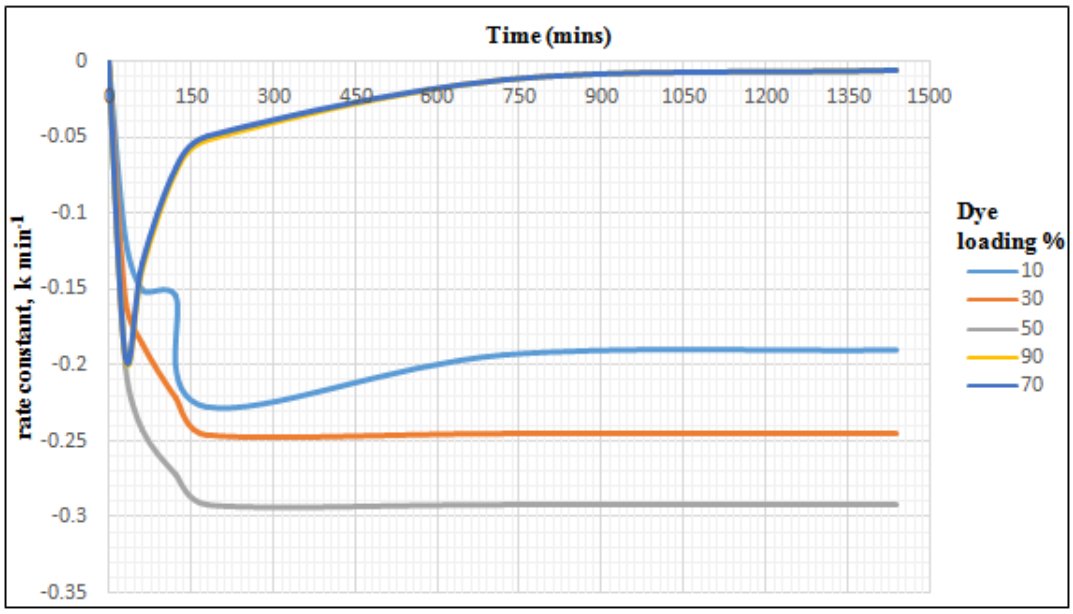

Figure 5: This plot represents the deviating nature of rate constant with time, depicting an optimum dye loading% which is 50%.

From the graphs, optimum dye loading% and time was determined to be 50% and 180 mins respectively. So, possibly use of more amount of dye or more amount of dye loading does not mean that BC would be able to take up or absorb more amount of dye, after it reaches its saturation point, no further dye particles were allowed to get inside the microstructure regardless of their loading%. [10]

IV. 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 apacity of bacteria such as A.xylinum present in SCOBY to produce cellulose has not reachedfullexpression,although reportsonindustrialproductiondo indicateanultimate potentialthat isboth greaterand more obtainablethan expected. This study projects an effective approach of determining the dye retention capacity of bacterial cellulose. Bacterial cellulose is readily used by the food industries to encapsulate various nutraceuticals and food supplements. By absorbing the anthocyanin, cellulose produced by kombucha tea shows an effective utility on absorbing anti-oxidant components.

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