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Optimization of food-grade bacterial cellulose production in raisin finishing side-stream extracts and synthetic media: Effect of citric, gallic, and ascorbic acid addition

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Introduction

Figure 1: Bacterial cellulose from *K. sucrofermentans*.

Bacterial cellulose (BC) is an extracellular microbial polysaccharide that can be produced by a large group of microorganisms with the bacterium *Komagataeibacter sucrofermentans* being established as the model microorganism for its production because of its high BC yields and the ability to utilize a variety of C- and N-sources. Despite its numerous applications, the high cost of BC production in synthetic substrates still makes its applications ineffective. For that reason, researchers and industries are in seek of efficient production methods featuring low-cost substrates (like **agri-food wastes**, **by-products** & **side-streams**). The industrial finishing of Corinthian currants (black raisin variety cultivated in Greece) generates a large amount of side-stream (**5-6% of the raw material**), with similar nutritional quality with the marketable currants. This **finishing side-stream (FSS)** has been proposed as substrate for food-grade BC production. Apart from tartaric acid which is the main acid in FSS, common organic acids and phenolic compounds in food wastes and side-streams, are **citric acid**, **ascorbic acid** (**citrus wastes**) and **gallic acid** (**FSS, grape wastes**, **tea extracts**, and a variety of other plant sources). These compounds are known to affect the production of BC.

In this study, the combined effect of ascorbic, citric, and gallic acid on BC biosynthesis in FSS extracts was studied, in comparison with synthetic media. Optimization of BC production in the substrates was effected by **Response Surface Methodology (RSM)** based on the **Central Composite Design (CCD)** combining the above agents, in order to predict the optimum composition of a low-cost natural substrate made by mixing various agri-industrial side-streams or wastes.

Experimental

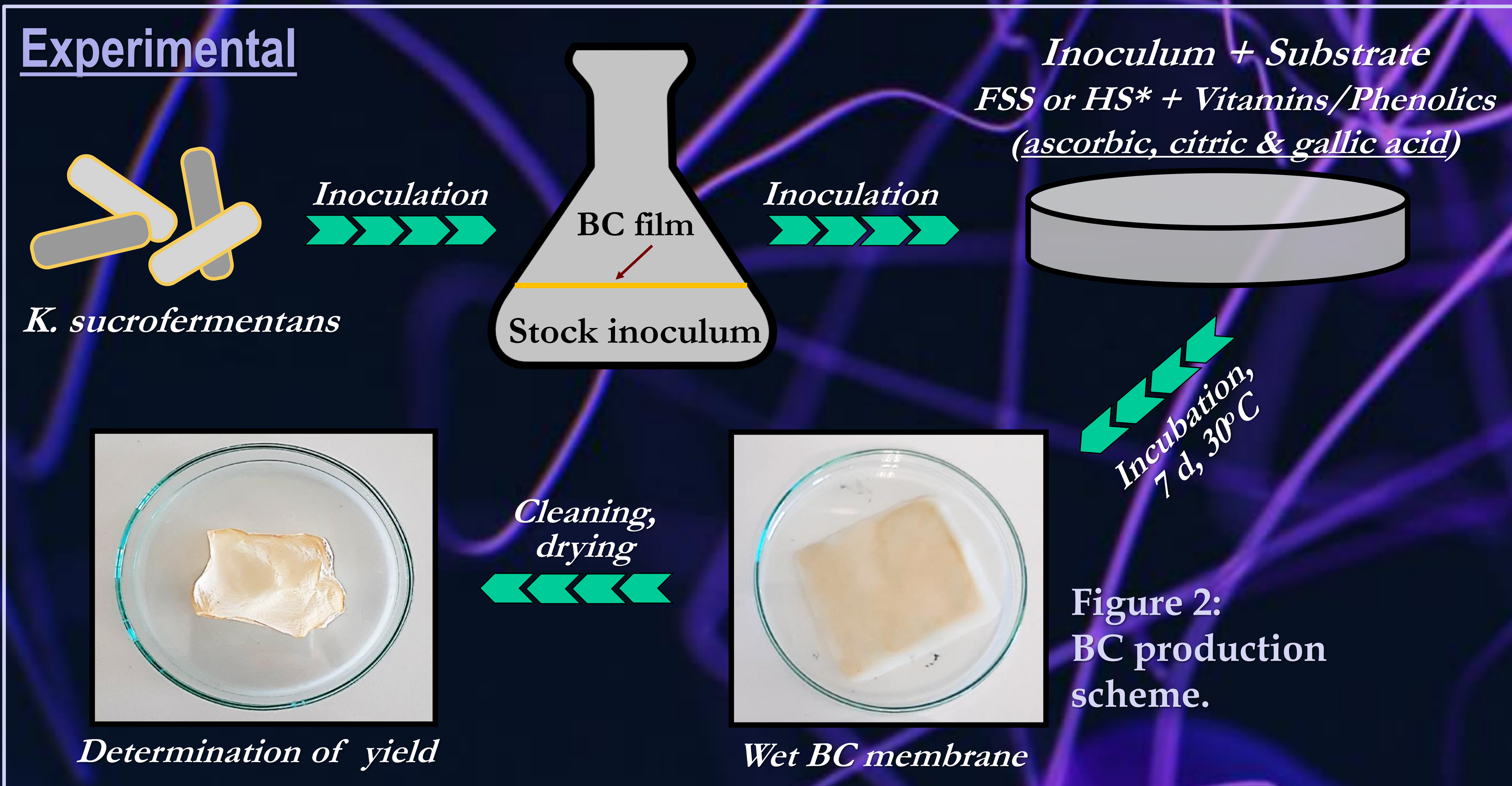
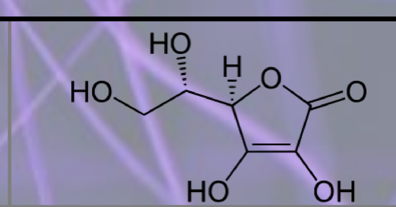
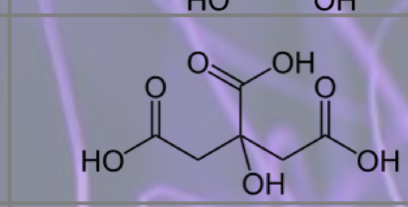
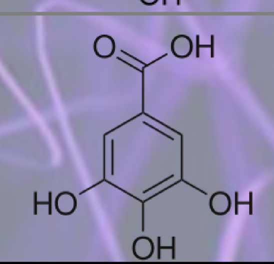


Figure 2: BC production scheme.

Table 1. Independent variables and their coded values, for RSM/CCD optimization of BC production.

Independent Variables (Concentration, g/L)		Symbol	Coded Values		
			-1	0	1
Ascorbic acid		X_1	0.00	5.00	10.00
Citric acid		X_2	0.00	0.50	1.00
Gallic acid		X_3	0.00	1.00	2.00

***HS medium (%w/v):** glucose 2.0, bacterial peptone 0.5, yeast extract 0.5, Na_2HPO_4 0.27, citric acid 0.115, in water; **FSS extract:** FSS+70 ℃ water (1:1) until an extract of 4 °Be density is received.*

Results & Discussion

2nd-order linear regression equations were obtained, which describe the relation between the dependent variable and the independent variables for each substrate:

For HS medium: BC yield (g/L) $= 5.110520 - 0.278873X_1 + 0.164758X_2 + 1.431640X_3 - 0.017833X_1X_2 - 0.020250X_1X_3 + 0.044167X_2X_3 + 0.022327X_1^2 - 0.047475X_2^2 - 0.611818X_3^2$

For FSS extract: BC yield (g/L) $= 15.359950 - 0.707555X_1 - 8.554550X_2 + 2.529230X_3 - 0.210000X_1X_2 + 0.088500X_1X_3 + 0.050000X_2X_3 + 0.012545X_1^2 + 6.994550X_2^2 - 2.051360X_3^2$

The **predicted values** of the BC yield using the above optimal combination of factors (**citric acid 0.5 and gallic acid 1.0 g/L for FSS, and 1.0 and 2.0 g/L for HS, respectively**) in the mathematical model were **13.33 g/L for FSS** and **5.86 g/L for HS**. **Confirmation** of these values was done by repeating the experiment with the best obtained factor values. Specifically, 3 experiments were performed and the obtained BC yields were found to be even higher: **15.13±0.05 g/L for FSS** and **6.20±0.01 g/L for HS**. On the other hand, the yield of BC production in **plain FSS extract** was **11.60±0.00 g/L**, and in **plain HS medium** was **5.40±0.01 g/L**.

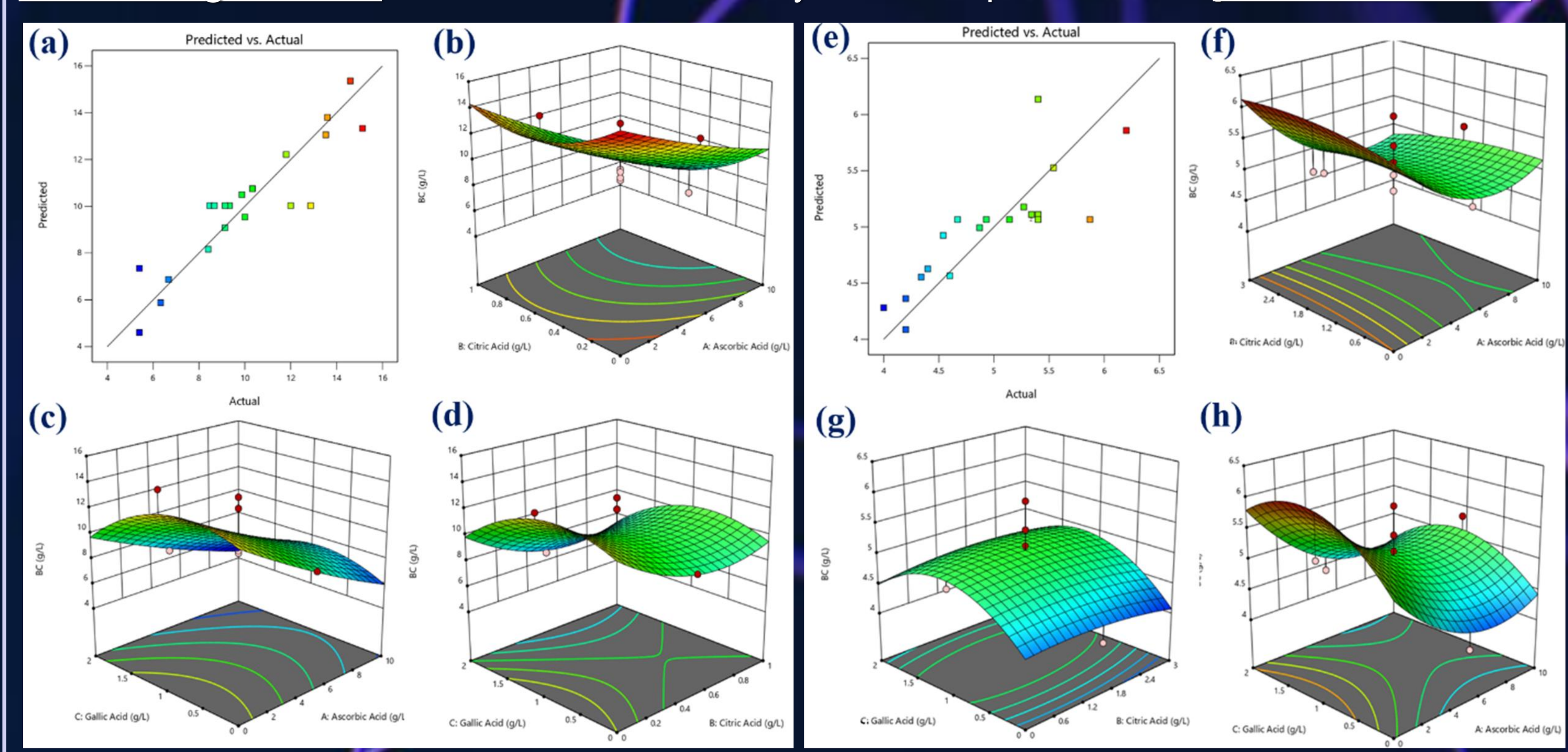


Figure 3: (a) Predicted values against experimental data of BC yield in FSS extract (g/L) according to the experimental design, and 3D-imaging of the BC yield response surface at varying concentrations (g/L) of: (b) citric acid and ascorbic acid, (c) gallic acid and ascorbic acid, and (d) citric acid and gallic acid. (e), (f), (g) and (h) are the corresponding graphs for HS-medium).

Conclusion

The BC production is affected by the addition of citric acid, gallic acid and ascorbic acid). The results will help towards the development of low-cost substrates for efficient food grade BC production from **FSS** with suitable additions of **waste citrus juice** and/or **tea extracts**, or **other waste biomass** sources containing the studied factors.

Images: Wikipedia, Public domain

Citric-, gallic-, and ascorbic acid-rich food waste

References:

[1] **Bekatorou et al. 2019**. Bacterial cellulose production using the Corinthian currant finishing side-stream and cheese whey: Process optimization and textural characterization. *Foods*, 8(6), 193; [2] **Fernandes et al., 2020.** *Polymer Engineering & Science*, 60(11), 2814-2826. [3] **Keshk, 2014.** Vitamin C enhances bacterial cellulose production in Gluconacetobacter xylinus. *Carbohydrate Polymers*, 99, 98-100.

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