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# Optimization of food-grade bacterial cellulose production by *Komagataeibacter sucrofermentans* in raisin finishing side-stream extracts and synthetic media: Effect of vitamins and phenolic compounds

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## Introduction

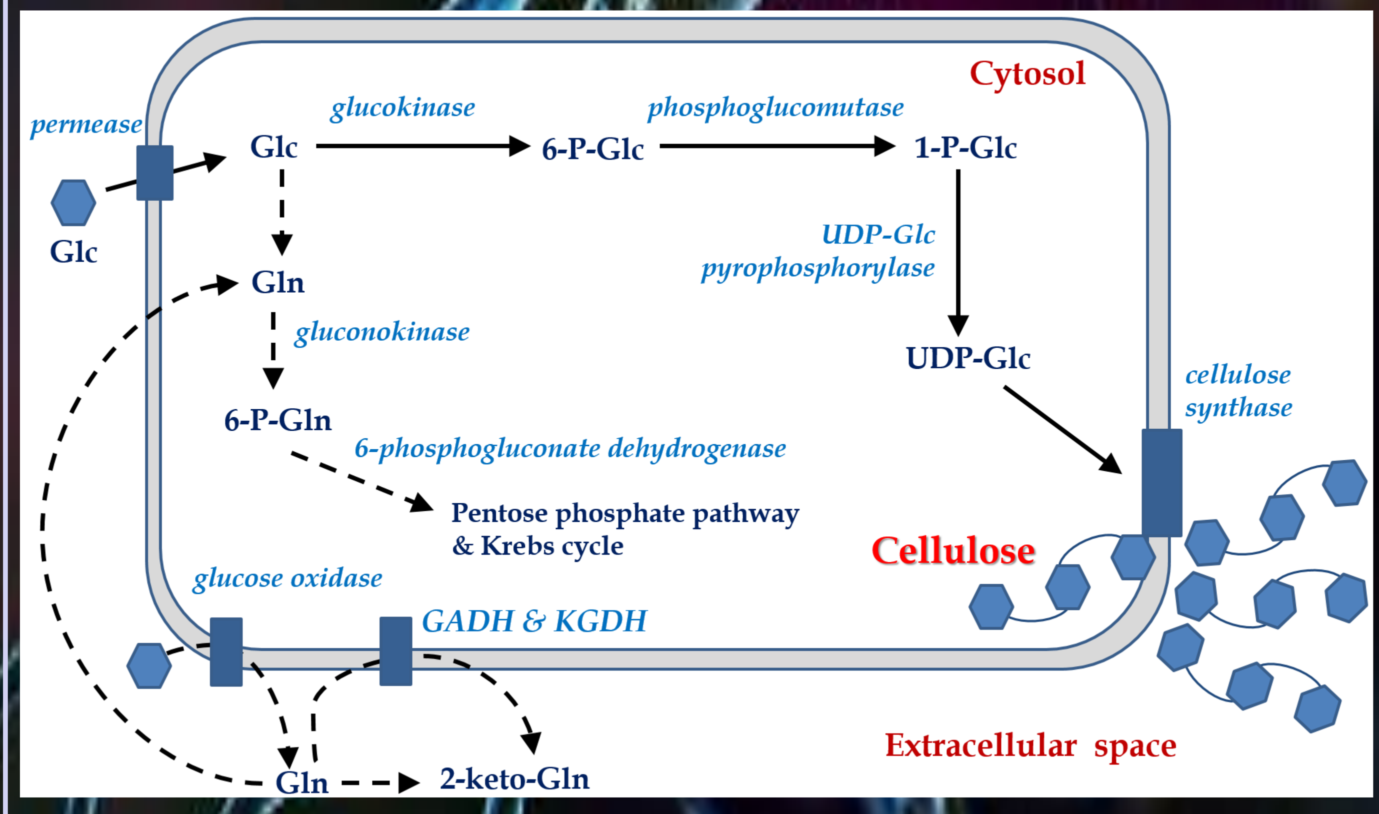


Figure 1: BC production in *K. sucrofermentans*

**Bacterial cellulose (BC)** is a microbial nano-fibrous product (**highly polymerized β-1→4 glucan**), with versatile applications in the food, materials, pharmaceutical & medical sectors. It is produced by a variety of microorganisms, with ***Komagataeibacter sucrofermentans*** being the model bacteria for both scientific studying and large-scale synthesis. The porosity of its matrix, along with its relatively simple cleaning and modification procedures, make it an ideal material for biotechnological applications. Among low cost raw materials for BC production, the **finishing side-stream (FSS) of Corinthian currants** (black raisins cultivated in Greece) has been successfully proposed as substrate for food-grade BC production. Also, **vitamins** such as **ascorbic acid** and **thiamine** and **phenolic compounds** such as **gallic acid**, have been found to enhance BC production and affect its textural properties. Their combination in FSS extracts or in synthetic media for BC production by *K. sucrofermentans* **has not been reported**, and is the aim of the present study. Optimization was effected by **Response Surface Methodology (RSM)** based upon the **Central Composite Design (CCD)**, in order to predict the **optimum mixing of low-cost substrates** based on agri-industrial side-streams or wastes (containing these factors) for efficient, low-cost BC production.

## Experimental

Figure 2: BC production scheme.

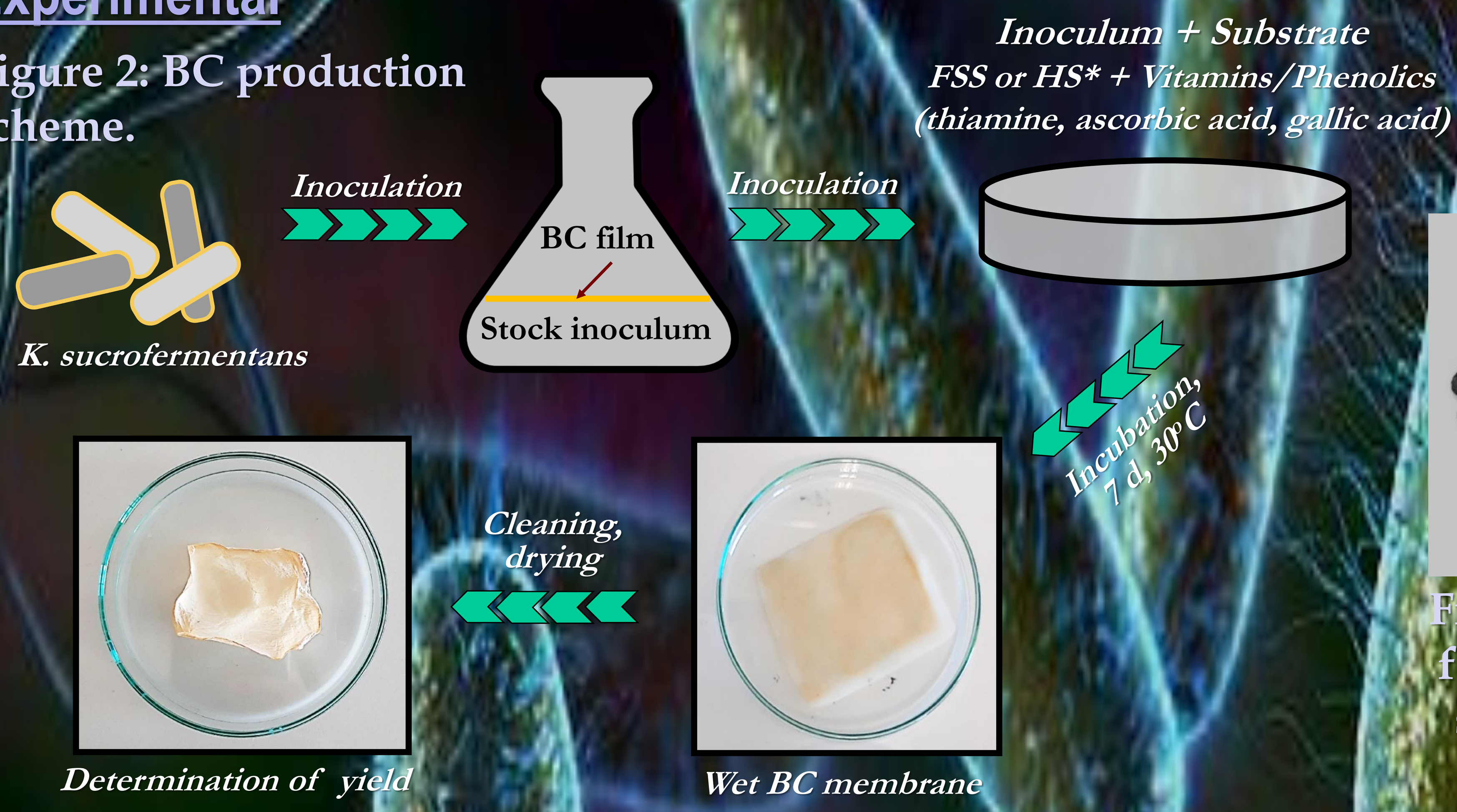


Figure 3: Raisin finishing side-stream (FSS).

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

| Independent Variable (Concentration) | | Symbol | Coded Values | | |
|---|---|---|---|---|---|
| | | | -1 | 0 | 1 |
| Ascorbic acid | g/L | $X_1$ | 0.00 | 5.00 | 10.00 |
| Thiamine | g/L | $X_2$ | 0.00 | 0.04 | 0.08 |
| Gallic acid | g/L | $X_3$ | 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°C water (1:1) until an extract of 4°Be density is received.*

## Results & Discussion

**2nd-order linear regression equations** were obtained form the RSM/CCD, which describe the relation between the dependent and the independent variables:

**For the HS medium**: BC yield (g/L) = $2.081230 - 0.003727X_1 + 5.159090X_2 - 0.955636X_3 + 0.212500X_1X_2 - 0.011500X_1X_3 + 1.437500X_2X_3 - 0.005727X_1^2 - 151.988640X_2^2 + 0.426818X_3^2$

**For the FSS extract**: BC yield (g/L) = $15.087410 + 0.172591X_1 + 136.361360X_2 - 0.497045X_3 - 1.750000X_1X_2 - 0.033500X_1X_3 - 23.750000X_2X_3 - 0.056509X_1^2 - 1048.579550X_2^2 + 0.587273X_3^2$

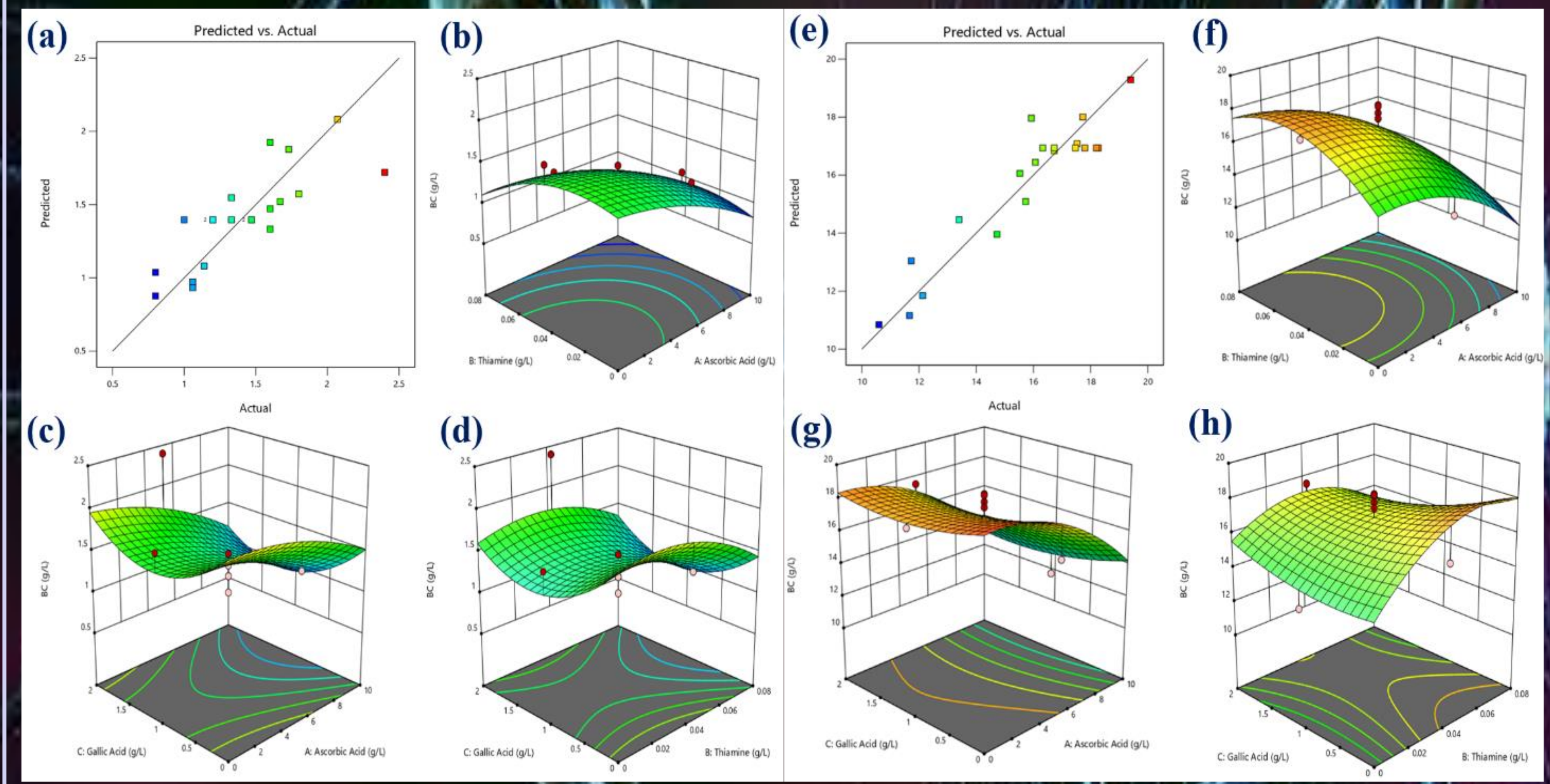


Figure 4: (a) Predicted values against experimental data of BC yield (g/L) in synthetic HS-media, according to the experimental design, and 3D-imaging of the BC yield response surface at varying concentrations (g/L) of (b) thiamine, ascorbic acid, (c) gallic acid, ascorbic acid, & (d) gallic acid & thiamine. (e), (f), (g) and (h) are the corresponding graphs for the FSS extract).

The **predicted value** of the BC production yield using the above optimal combination of factors in the mathematical model (**ascorbic acid 0.5, thiamine 0.04, and gallic acid 2.0 g/L for HS**, and **0.0, 0.08, and 0.0 g/L for FSS**, respectively) was **2.08 g/L for HS** and **19.29 g/L for FSS**.

**Experimental 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 (**2.40±0.00 g/L for HS** and **19.40±0.01 g/L for FSS**).

On the other hand, the yield of BC production in the **plain HS medium**, without the addition of the above factors, was **1.07±0.01 g/L**, and in **plain FSS extract** it was **15.73±0.02 g/L**.

## Conclusion:

BC production is generally affected by the addition of **vitamins** (ascorbic acid and thiamine) and **phenolic compounds** (gallic acid). However, for production in FSS extracts, **only thiamine had a significant effect**. The results will help towards the **development of cheap substrates** that contain vitamins and phenolic compounds, such as **grape and citrus waste**, for efficient BC production, leading to the development of **BC-based functional foods** (e.g. prebiotics/synbiotics), **added value in the industrial sector**, and **economic benefits for the local agricultural communities**.

## 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] **Lestari et al., 2014.** Study on the production of bacterial cellulose from *Acetobacter xylinum* using agro - waste. *Jordan J Biol Sci* 7(1), 75-80. [3] **Fernandes et al., 2020**. Hybrid bacterial cellulose-collagen membranes production in culture media enriched with antioxidant compounds from plant extracts. *Polymer Eng Sci*, 60(11), 2814-2826. [4] **Keshk, 2014**. Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. *Carboh Polym* 99, 98-100.

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