

BIOCHEMISTRY OF MICROORGANISMS

<https://doi.org/10.30970/apmicro.2026.36>

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MODULATION OF BACTERIAL CELLULOSE PRODUCTION BY THE KOMBUCHA SYMBIOTIC COMMUNITY THROUGH INOCULUM RATIO AND METABOLIC SUPPLEMENTATION

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Abstract. This study investigated how the fresh tea medium-to-SCOBY (symbiotic culture of bacteria and yeast) inoculum ratio and supplementation with α -ketoglutaric acid (α -KG), citric acid, magnesium sulfate, and glycerol affect bacterial cellulose (BC) biofilm formation, medium pH, and total polyphenol content over 14 days of static fermentation. α -KG and citric acid, both TCA cycle intermediates, produced qualitatively similar biphasic dose-response effects on BC yield: moderate supplementation doubled biofilm mass, whereas high doses were inhibitory. MgSO_4 and glycerol both reduced BC yield. Thus, α -KG and citric acid, at defined moderate concentrations, are promising low-cost supplements for enhancing BC yield in kombucha-based systems.

Keywords: bacterial cellulose, alpha-ketoglutaric acid, citric acid, kombucha.

Bacterial cellulose (BC) is a biopolymer produced by several bacterial species and has attracted considerable attention as a sustainable alternative to conventional textile materials, which often require energy-intensive processing and harsh chemical treatments. Among BC-producing microorganisms, acetic acid bacteria belonging to the genera *Komagataeibacter*, *Acetobacter*, and *Gluconobacter* are particularly well characterized (El-Gendi et al., 2022). More recently, increasing attention has been directed toward the use of complex microbial communities, including the symbiotic cultures associated with kombucha, as potential platforms for BC production (Gagliardi et al., 2025). Kombucha is a fermented beverage produced through the metabolic interactions of a symbiotic culture of bacteria and yeasts (SCOBY), which typically includes acetic acid bacteria, lactic acid bacteria, and various yeast species. Previous studies have demonstrated that supplementation with specific metabolites and nutrients, including ethanol, glycerol, organic acids such as acetate, ascorbic acid, and nitrogen sources, can enhance BC production by kombucha-associated or kombucha-derived *Komagataeibacter* strains (Li et al., 2012; Agustina et al., 2025). While traditional optimization strategies for BC largely focus on upstream carbon sources like glycerol or ethanol, downstream metabolic regulators remain poorly understood.

In the present study, alpha-ketoglutaric acid (α -KG), citrate, and Mg^{2+} were selected as kombucha supplements because they influence distinct metabolic processes that may affect SCOBY-mediated bacterial cellulose formation.

A commercial kombucha starter (SCOBY) purchased from a local market in Ivano-Frankivsk (Ukraine) in 2024 was used in the experiments. The SCOBY starter was cultured

in the sweetened tea infusion to create stock cultures. The sweetened tea infusion was prepared by mixing boiled water, Monomax “Ceylon” black pekoe tea (1 tea bag per 700 mL), and sucrose (50 g/L). In the first experiment, the effects of the fresh tea-to-SCOBY inoculum ratio were determined. In the next experiment, the tea infusion was divided into several portions, supplemented with the tested compounds, and mixed with the parental culture (kombucha SCOBY inoculum). Independent experiments were conducted to evaluate the effects of supplementation with citric acid, α -ketoglutaric acid (α -KG), magnesium sulfate (MgSO_4), and glycerol on kombucha fermentation and bacterial cellulose production. The wet and dry masses of the BC biofilms formed were assessed on day 14. The acidity of the medium was measured with a pH meter at the beginning of cultivation (day 0) and then on day 14. Total polyphenols were measured using the Folin-Ciocalteu colorimetric method.

A higher medium-to-inoculum ratio (2:1) yielded significantly more wet and BC biomass than a lower ratio (1:2), and this ratio was selected as the baseline conditions for further experiments. Both α -KG and citric acid, tricarboxylic acid cycle intermediates, showed biphasic, dose-dependent effects on BC yield: moderate supplementation (0.25 g/L α -KG; 0.5 g/L citric acid) approximately doubled biofilm mass relative to control, while higher doses markedly suppressed or abolished BC growth, with α -KG showing stronger inhibition at high concentration than citric acid. α -KG supplementation also modestly reduced total polyphenol content in kombucha in a dose-dependent manner. In contrast, MgSO_4 (0.8 g/L) and glycerol (40 g/L) both significantly decreased BC yield relative to the control, and glycerol also reduced polyphenol content. Medium pH declined over cultivation in all groups regardless of treatment, indicating that supplementation affected cellulose biosynthesis rather than the overall acidification dynamics of fermentation.

The results support α -KG and citric acid, applied within a narrow, optimal concentration range, as practical, low-cost strategies for improving BC yield in kombucha fermentation, and also underscore the importance of dose optimization when implementing supplementation strategies. With regard to magnesium sulfate and glycerol, the unexpected reduction in yield observed in this study warrants “dose-response” studies in the tea-based SCOBY system, since neither compound was tested across a wide range of concentrations.

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