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Optimizing productivity and quality of bacterial cellulose by applying intermittent harvests

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Bacterial cellulose (BC) is a natural biopolymer produced by a wide variety of bacteria as a fibrous structural component of biofilms. Unique and versatile properties of BC make it a promising biomaterial with application in various fields (e.g. biomedical, textile and technological industries). However, for a large-scale industrial use it is necessary to increase BC productivity and decrease production cost. Traditionally, BC is produced by static fermentation culture in which cellulose is formed as a biofilm pellicle at the air-liquid interface. However, once cellulose reaches a certain thickness, both cell growth and BC production stop because biofilm cells lack nutrients and planktonic cells lack oxygen. Hence, in this study we investigate an alternative strategy for cellulose production by applying intermittent harvesting under static conditions. Each cellulose harvest forces planktonic cells within the media to re-colonize the air-liquid interface and form a new BC film and this was repeated weekly for up to 92 days. Using this strategy, the productivity is enhanced over time with up to 3-fold increase over the 35 first days (625.63□49 g/L compared to 201.9□ 1.06 g/L wet weight). Refilling medium allowed us to extend the incubation time and better understand how BC physical properties evolve as a function of growth condition and harvest sequences. Indeed, X-ray diffraction experiments have shown an evolution of cellulose crystallinity and crystallite size throughout long term incubation and the intermittent harvesting sequences. Late cellulose harvests were characterized by increased crystallinity index (CI) and enhanced stiffness of the material. Towards the end of the experiment, we have isolated a cellulose producing G. hansenii variant, M2, that had a 1.7-fold higher productivity but showed a lower crystallinity and elastic modulus than wild type (WT). Environmental scanning electron microscopy depicts distinct differences in matrix architectures with the M2 strain cellulose consisting of layers with much higher inter-layer distances compared to the WT cellulose.

In conclusion, BC harvest strategy can be considered as a profitable alternative to increase productivity, generate tunable cellulosic materials and lead to the emergence of novel mutants that produce cellulose with quite unique physical and mechanical properties.