Optimisation and Characterisation of Bacterial Cellulose produced by *Gluconacetobacter xylinus*

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Abstract: Bacterial cellulose (BC) presents a highly porous and crystalline nanofibrillar structure due to its high water retention capacity, hydrophilicity and excellent mechanical properties and is therefore suitable for various biomedical applications. However, a high production cost and low-yield has limited the large-scale production of BC and its commercial application. Optimisation of the bioprocess is important for the scale-up of the BC production. In our present study, we optimised BC production by *Gluconacetobacter xylinus* under static-conditions using different growth media and culture conditions. We further characterised the physical and chemical properties of BC by SEM, XPS and FT-IR analysis.

BC was produced by the Gram-negative bacteria, *Gluconacetobacter xylinus* (NCIMB 5346) in a static fermentation under various tested static fermentation conditions.

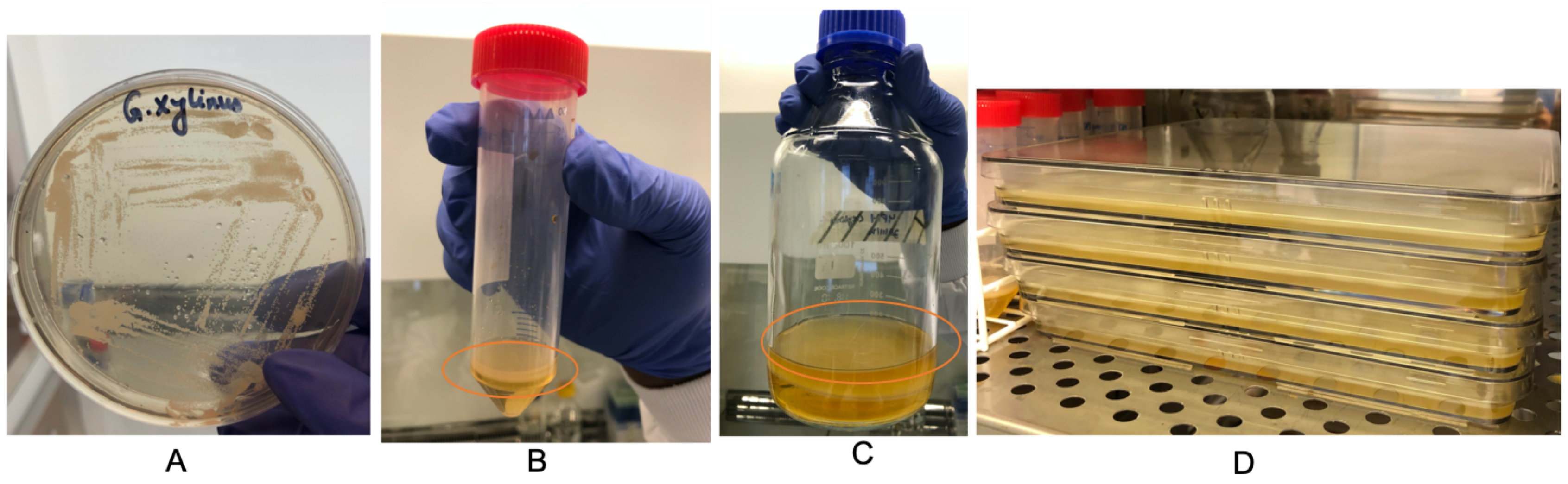

Fig. 1 Microbial cellulose production using the static culture method. (A) *Gluconacetobacter xylinus* grown on HS agar plate (B) Pellicles were observed after two days of inoculation of a single colony in a 5ml HS and YPM medium (C) Pellicle formation under static conditions of cultivation in 1L Duran bottles and (D) Tissue culture trays containing YPM medium after 7 days.

BC pellicles produced during the fermentation process were manually harvested to remove to any cell biomass.

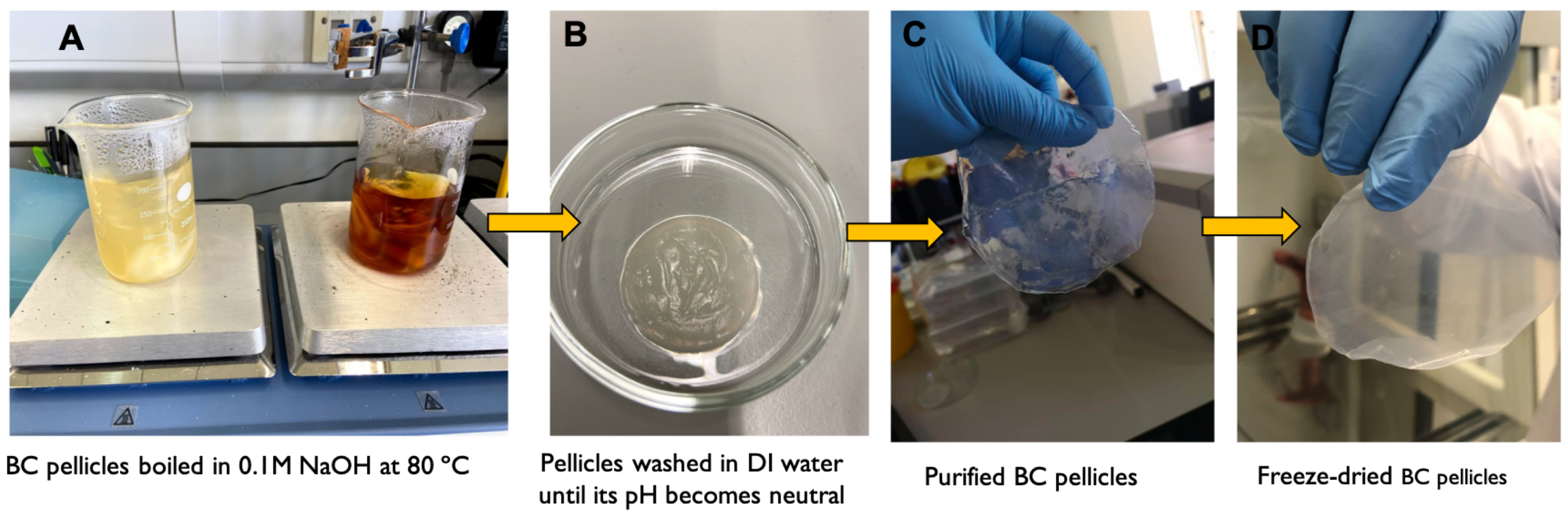

Fig. 2 (A) Pellicles purified using 0.1M NaOH at 80 °C (B) Samples rinsed with deionised water to reach neutral pH and until the pellicle became transparent (C) a purified BC pellicle. (D) Dried BC pellicle.

The polymer yield for the biosynthesis as well as the productivity was evaluated by measuring the weight of the wet pellicles.

Type of culture medium	Time (days)	Volume of medium (mL)	Mass of wet pellicle (g)	Titre (gL^{-1})
HS in tray	7	200	132.37 ± 8.02	661.86 ± 40.11
HS in bottle	7	200	29.5 ± 3.89	147.46 ± 19.45
YPM in tray	7	200	128.92 ± 16.02	644.6 ± 80.1
YPM in bottle	7	200	35.7 ± 3.80	176.3± 19.03
GYC in bottle	12	200	4.18 ± 0.14	20.9 ± 0.70

Table. 1 Yield of bacterial cellulose under static condition of cultivation using various media.

The scanning electron micrograph (SEM) of BC showed the ultrafine microfibril in a well interconnected network structure. The nanosized thread like microfibrils were tightly packed and densely woven with each other.

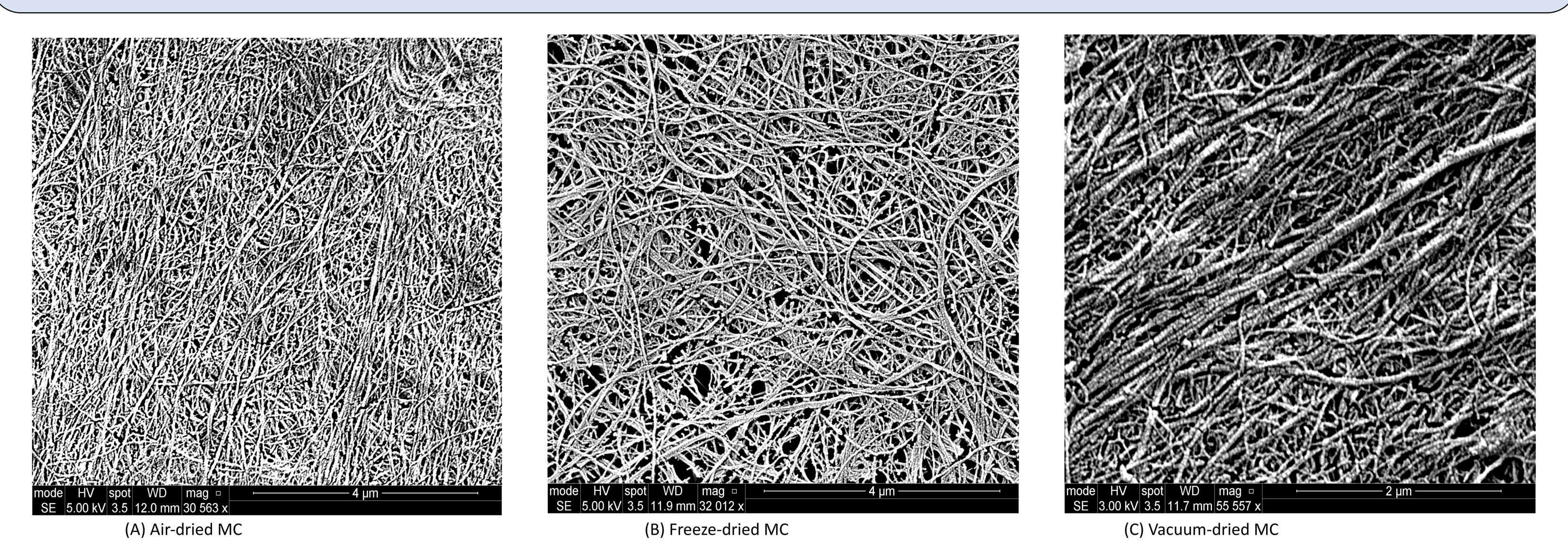

Fig. 3 SEM images of (A) air-dried (B) freeze-dried and (C) vacuum-dried sample.

The FTIR spectrum peak of the BC pellicles in the region of 3344 cm^{-1} characterizes the stretching vibrations of OH groups, the peak in the region of 2892 cm^{-1} indicates the stretching vibrations of the C–H groups. In addition, several typical bands for bacterial cellulose were observed in the region of 1000–1200 cm^{-1} due to C–O and C-O-C vibrations

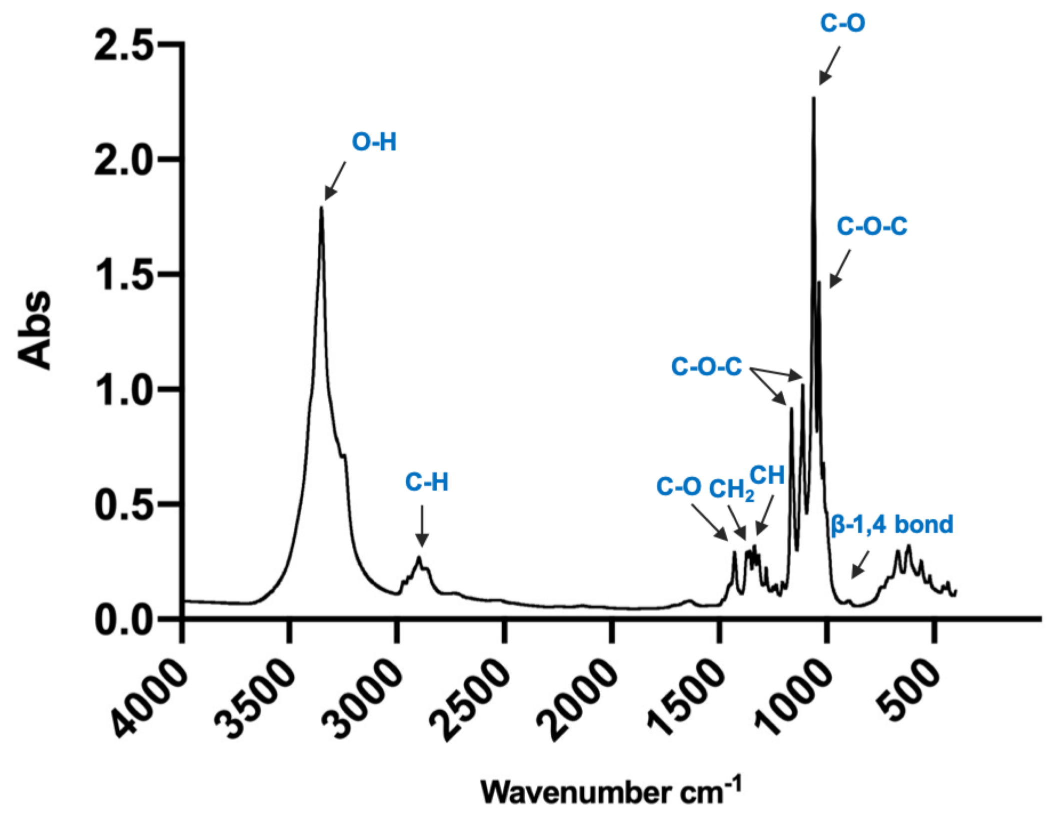

Fig. 4 FT-IR spectra of microbial cellulose .

A quantitative indication of the surface elemental composition of the microbial cellulose was obtained through XPS spectroscopy. The survey XPS scans indicated the elements present on the surface with carbon and oxygen, being the elemental constituents of cellulose.

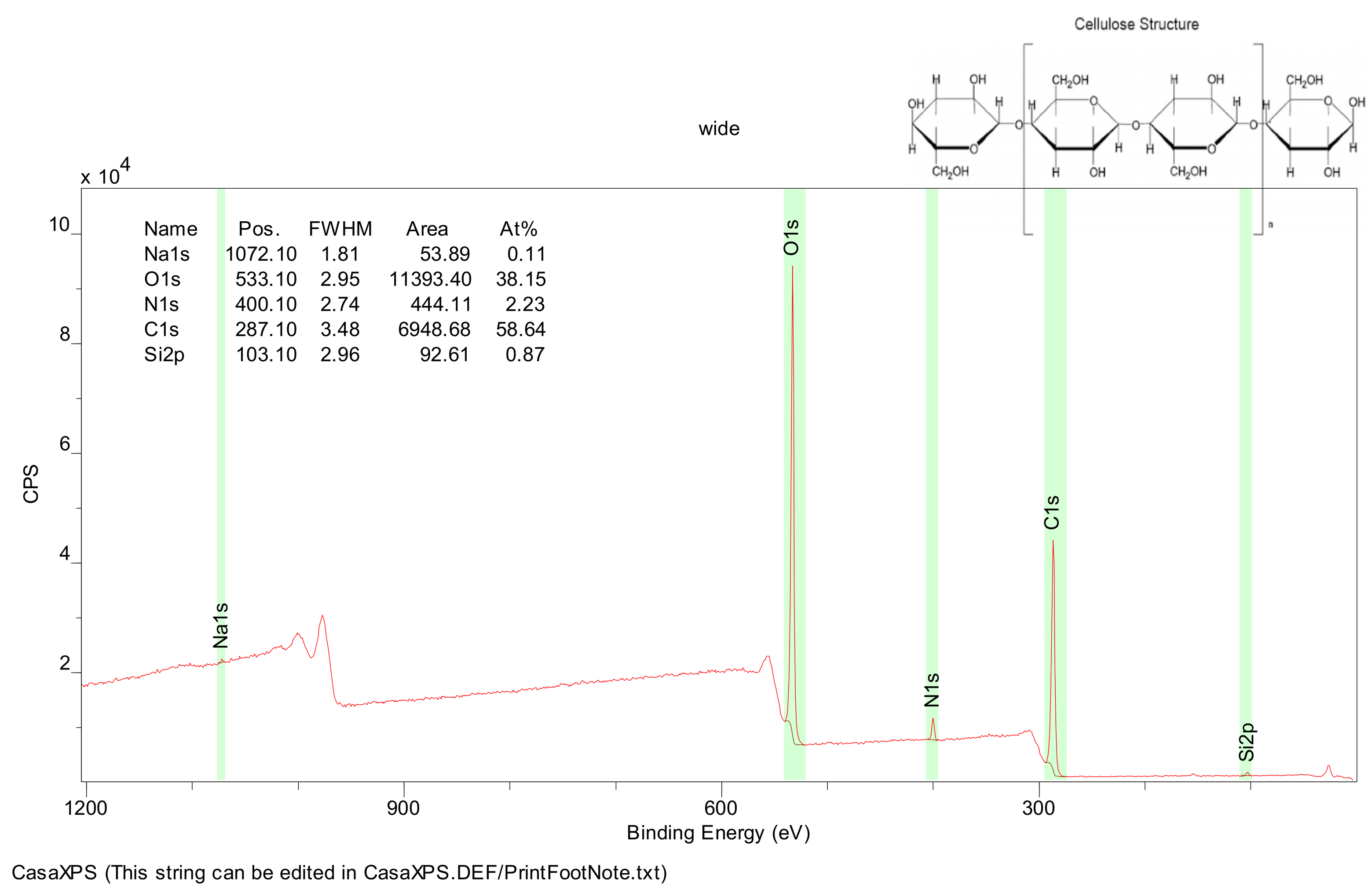

Fig. 5 XPS survey scan of microbial cellulose .

Summary

- BC production was optimised using *Gluconacetobacter xylinus* under static-conditions using different growth media and culture conditions.
- The optimised culture conditions resulted in BC yield of 662 gL^{-1} wet weight which was comparatively higher than previously reported yields of BC by *G. xylinus*.
- The SEM analysis of the dry pellicles showed an ultrafine microfibrillar structure. BC was chemically characterized by XPS and FT-IR analysis.
- The rheological measurements showed that the material behaved as a hydrogel with a viscoelastic property.
- Due to its sustainability, biodegradability, purity, biocompatibility and non-cytotoxicity the cellulose produced can be used in green packaging applications, tissue engineering and biomedical applications.

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