BIOTECHNOLOGY for A CIRCULAR BIOECONOMY

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Valorization of coffee agro-industry residues for prebiotic production by one-pot fermentation

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CENTRE OF BIOLOGICAL ENGINEERING

Abstract

- Prebiotics are interesting compounds able to modulate the gut microbiota by inducing the growth/activity of beneficial bacteria in the GI tract, while inhibiting pathogens
- Xylooligosaccarides (XOS) are the only prebiotics that can be produced from lignocellulosic biomass, e.g., from inexpensive, abundant and renewable agro-residues, which is encouraging to the food ingredient industries
- Using coffee agro-industry residues for XOS production through a sustainable process is aligned with the concept of circular economy
- In this work, the potential microbial production of XOS from coffee silver skin (CSS) and CSS pellets (CP) was evaluated, using one-pot fermentation and a recombinant *Bacillus subtilis* 3610. Previously, this strain was genetically modified to express the xylanase gene (*xyn2*) from *Trichoderma reesei* (*B. subtilis_xyn2*)
- CP presented the highest potential for XOS production. After process optimization, the highest reducing sugars yield (Y_{RS}), 63 ± 3 $mg.g_{CP}^{-1}$, was achieved at 8 h, 45 °C, pH 7.0 and 10 $g.L^{-1}$ of CP
- One-pot fermentation proved to be a promising and advantageous strategy for XOS production from CP, as compared to the use of commercial enzymes
- This work provides important insights for novel bioprocess integration approaches using agro-residues towards production cost reduction

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CSS
Coffee Silver Skin

CP
CSS pellets

Chemical characterization
(NREL/TP-510-42618-42622-4218 protocols)

↓

Autoclave sterilization
(121 °C, 15 min)

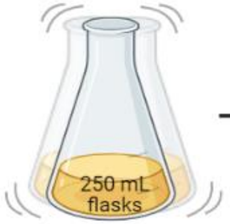
↓

B. subtilis_xyn2
LBAgar medium
(overnight growth, 37 °C)

↓

Pre-inoculum
(4 mL LB medium, 1 h, 40 °C, 250 rpm)

→



One-pot Fermentation
(basal medium Vogel 2% (w/v))

→ **CP Residue selection** → **Process optimization** (CP concentration, temperature, pH) → **Analytical Techniques** (Thin Layer Chromatography, 3,5-dinitrosalicylic acid method, High-performance liquid chromatography)

Challenge: *To evaluate the potential microbial production of XOS from CSS and CSS pellets (CP), using one-pot fermentation and a recombinant Bacillus subtilis 3610*

Experimental

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Results

Table 1 – Chemical characterization of Coffee Silver Skin (CSS) and CSS pellets (CP) in dry weight % (w/w)

% (w/w)	CSS	CP
Acid Soluble Lignin	4.2 ± 0.6	4.85 ± 0.05
Insoluble Lignin	18.1 ± 0.3	20.1 ± 0.3
Hemicellulose	15.5 ± 0.3	17.6 ± 0.6
Cellulose	18.9 ± 0.1	18.9 ± 0.4

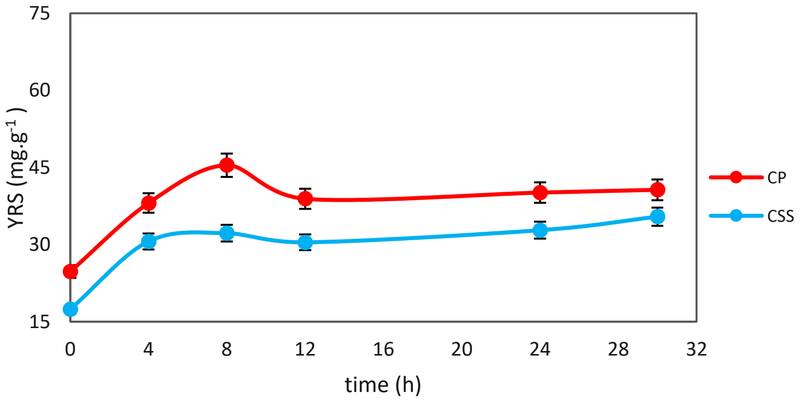

Fig. 1 – Reducing sugars production yield (YRS) obtained for *B. subtilis_xyn2* using CSS and CP at 20 g.L^{-1}, 45 °C, pH 7, 150 rpm.

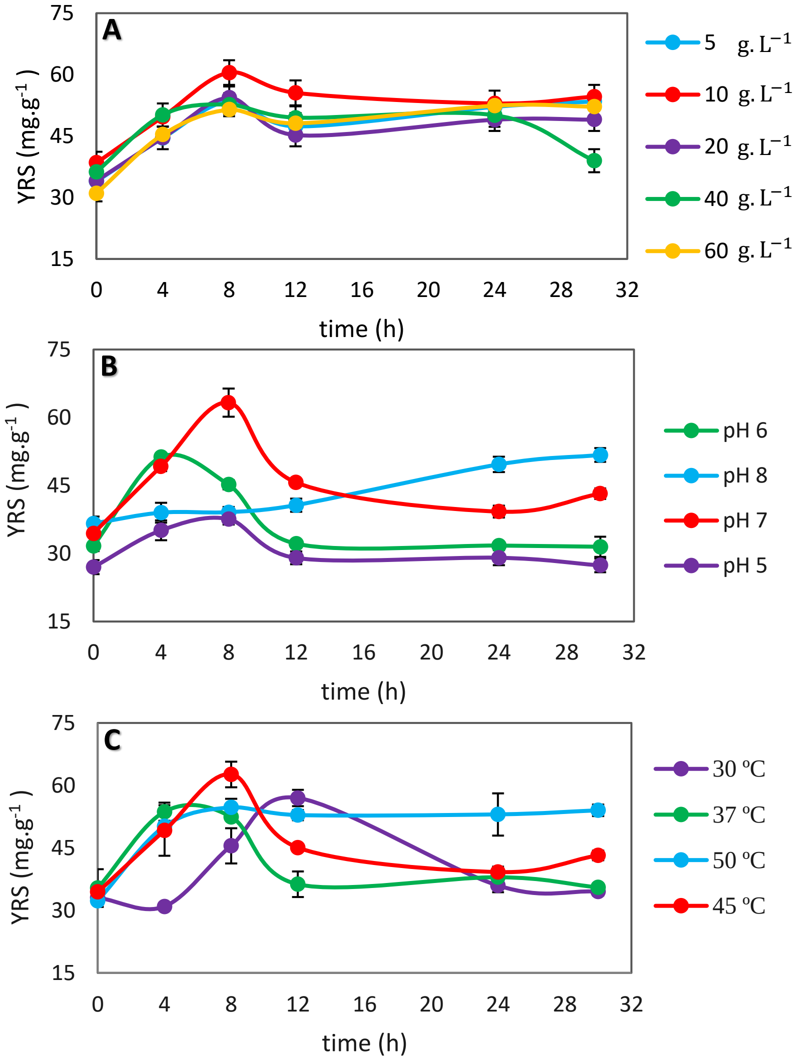

Fig. 2– Reducing sugars production yield (YRS) obtained for *B. subtilis_xyn2* using different: **(A)** CP concentration at 45 °C; **(B)** pH values at 45 °C and 10 g.L^{-1} of CP; **(C)** temperatures at pH 7.0 and 10 g.L^{-1} of CP.

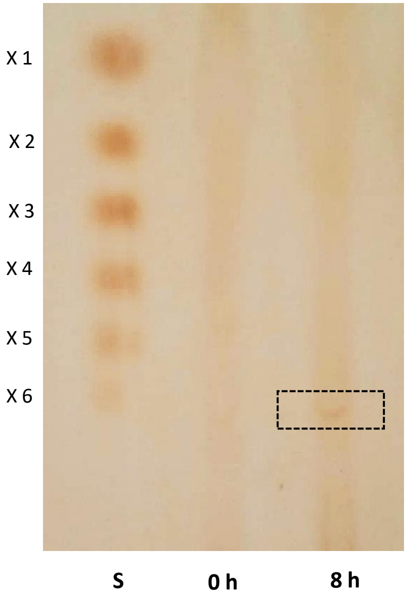

Fig.3 –TLC of the supernatants obtained from the one-pot fermentation of CP by *B. subtilis_xyn2* under optimal conditions at 0 and 8 h (optimal time). A mixture containing 2 g.L^{-1} of xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5) and xylohexaose (X6) was used as standard (S). Butanol:acetic acid:water

Conclusions

- CP presented the highest potential for XOS production.
- The highest reducing sugars yield (63 ± 3 mg.g$_{CP}^{-1}$) was achieved at 8 h, 45 °C, pH 7.0 and 10 g.L^{-1} of CP.
- The obtained sugar mixture presented a low amount of undesired free xylose (0,1824 g.L^{-1}) at the optimal time.
- One-pot fermentation proved to be a promising and advantageous strategy for XOS production from CP.

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