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Expanding the valorization routes of cheese whey: Lactose hydrolysis using *A. awamori*-derived β-galactosidase for the subsequent production of bacterial cellulose

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Abstract: Cheese whey constitutes one of the most polluting by-products of food industry. Regardless the numerous bioprocessing approaches that have been proposed for whey lactose utilization, still, valorization options are restricted by the fact that the majority of strains do not express the gene that encodes β-galactosidase. As a result, the formulation of several high value-added products is hindered, entailing at the same time definite end applications. The aim of this work was to undertake the cost-effective production of crude enzymes, including β-galactosidase, and the subsequent exploitation of whey hydrolysate in an upstream bioconversion process resulting in bacterial cellulose (BC) production. The ability of *Aspergillus awamori* to secrete β-galactosidase was evaluated via SSF using wheat bran as substrate. Specifically, β-galactosidase was assessed at 60-75 % initial moisture content. Crude enzyme extracts produced, were employed in whey hydrolysis at different temperatures (50-70°C) to estimate the effect of temperature in lactose hydrolysis. Subsequently, hydrolyzed whey was used for BC production by *Acetobacter xylinum*. Results demonstrate that β-galactosidase production was notably affected by moisture content and fermentation time, whereas the maximum activity of 148 U/g was observed at 70% initial moisture content after 79h of SSF. Hydrolysis kinetics showed a 93% lactose hydrolysis at 48h. The produced crude hydrolyzate was subsequently utilized in BC fermentations, leading to the production of up to 5.5 g/L of BC. Evidently, the above findings exhibit a novel and promising approach with respect to cheese whey hydrolysis, thereby expanding the output potential for end products.

Keywords: cheese whey; *Aspergillus awamori*; β-galactosidase; lactose hydrolysis; bacterial cellulose

1. Introduction

Cheese whey (CW) constitutes one of the most polluting by-products of food industry, owing to the high organic load. The exploitation of CW as carbon source, could be enhanced through utilization in fermentative bioconversions that generate diversified microbial metabolites (Lappa et al. 2019). The development of alternative bioprocesses to hydrolyze whey lactose as a low-cost onset material to obtain monomeric sugars, exhibits an emerging approach to valorize CW and investigate the potential to formulate previously overlooked added-value products, including biopolymers and more specifically bacterial cellulose. Within this context, microbial β-galactosidases have gained significant attention, featuring properties as high catalytic activity and reaction rate. Furthermore, many *Aspergillus* species have demonstrated the ability to secrete several enzymes using low-cost fermentation substrates (Martarello et al., 2019), thereby could provide an environmentally benign alternative to generate crude enzymatic consortia for further biocatalysis applications.

2. Material and Methods

2.1. Microorganisms

A. awamori strain 2B.361U 2/1 was kindly provided by Colin Webb (University of Manchester, Manchester, UK) and was employed for the generation of crude enzymes of β-galactosidase. *A. xylinum* strain 15973 purchased from DMS was used for bacterial cellulose production. Wheat bran (WB) employed in solid state fermentation (SSF) (26% carbohydrates, 14% proteins and 0.01% salt), was purchased from a local market. Cheese whey (~50g/L lactose) was provided by local dairy farms.

2.2. Experimental

SSF was performed in 250 ml Erlenmeyer flasks using 5g WB as a substrate, inoculated with 2 x 10^6 spores mL^{-1} fungal suspension. Fermentations were carried out at static conditions at 28 °C for approximately 3 days. The effect of temperature on hydrolytic activity of crude enzymes was evaluated at 50, 60, 65 and 70 °C at 48h. The hydrolysates were sterilized and pH value was adjusted to 6.0 and used for bacterial cellulose (BC) production. Experiment were conducted in 250 mL Erlenmeyer flasks containing 50 mL of hydrolysate. The media were inoculated with 10% (v/v) of 48 h bacterial sub-cultures, without any addition of nutrient sources and were further incubated at 30 °C on a 10 days static cultivation.

2.3. Analytical methods

Sugars concentration during hydrolysis and fermentation processes were quantified by High Performance Liquid Chromatography (HPLC) analysis (1200 series Agilent, USA). Moreover, free amino nitrogen (FAN) concentration was also determined in the hydrolysates and fermentation samples by the ninhydrin colorimetric method (Lie, S. 1973). Activities of β-galactosidase were measured by an o-nitrophenol-β-d-galactopyranoside (ONPG) assay (Raol et al., 2015).

3. Results

Figure 1 demonstrates the effect of initial moisture content (ranging from 60-75%), along with incubation time (1-5 days) on SSF. More specifically, the production of β-galactosidase reached a maximum of 148 U/g (db) at 70% initial moisture after 70-79 h of fermentation. The liquid crude enzymes obtained from SSF were used for CW hydrolysis. As presented in Figure 2, the optimum hydrolysis temperature was at 65°C, where almost all lactose content was hydrolysed. More specifically, hydrolysis kinetics showed a 93% lactose hydrolysis at 48h, using an initial crude enzymatic activity equal to 7.5 U/mL. Notably, hydrolysis time was significantly reduced by performing the hydrolysis with an initial enzymatic activity of 15 U/mL (data not shown). The produced crude hydrolyzate was subsequently utilized in BC fermentations, leading to the production of up to 5.5 g/L of BC, which is a remarkable concentration, taking into account previous cited research activities.

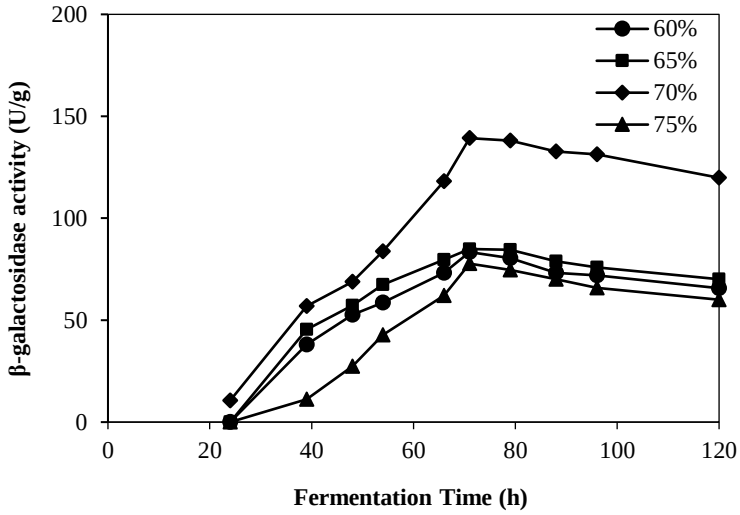

Figure 1. The effect of SSF moisture content on the production of crude β-galactosidase by *A. awamori*.

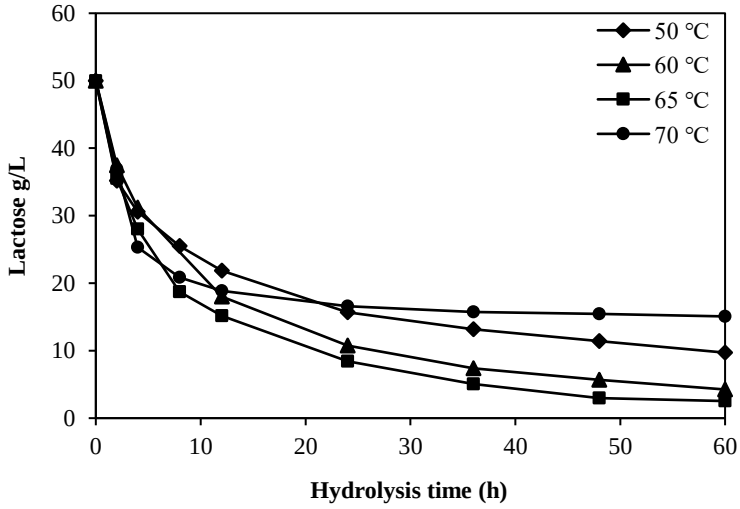

Figure 2. The effect of temperature on hydrolytic activities of crude β-galactosidase (7.5 U/mL) at ~50 g/L initial CW lactose content for 48h.

4. Conclusions

In the present study we demonstrated the significant lactose hydrolytic activity of a novel biocatalyst, aiming to generate cost-effective supplements for subsequent bio-composites production. Food industry by-products were also implemented within the context of developing cost-effective crude enzymes production, avoiding further purification steps. On top of that, significant BC production was achieved without the addition of further nutrients. The above findings exhibit a novel and promising approach in the context of cheese whey hydrolysis, thereby expanding the output potential for end products formulation.

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Conflicts of Interest: Page: 4

The authors declare no conflict of interest.

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