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Full biocatalytic synthesis of D-tagatose from whey permeate as a raw material

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PURPOSE OF THE ABSTRACT

D-Tagatose is an isomer of D-galactose and the epimer at C-4 site of D-fructose. It is one of the most promising functional sweeteners, similar to the polyols in having a low caloric value and tooth-friendly property; however, it has no laxative effect and it is able to modulate lipid metabolism, and reduce the symptoms associated with type 2 diabetes. Tagatose has the GRAS (Generally Recognized as Safe) status granted by the US Food and Drug Administration (FDA) since 2001, so it can be used in confectionery, beverages, health foods, and dietary products as a low-calorie, full-bulk sweetener. Elsewhere, it has entered in a phase 3 clinical trial, as a good candidate to treat type 2 diabetes. Tagatose does not increase insulin levels for glucose control.

This work was focused on the development of a totally biocatalytic methodology for obtaining D-tagatose, the best reaction conditions were studied for each step [1].

First step was the hydrolysis of whey permeate, and we chose the novel commercial ?-galactosidase from Bifidobacterium bifidum (Saphera), because this enzyme with lactose concentrations lower than 200 g/L has hydrolysis as its main reaction and shows negligible transgalactosylation [2]. This reaction was performed with concentrated whey permeate at 45 °C and 3.75 units/mL of enzyme for 3 h.

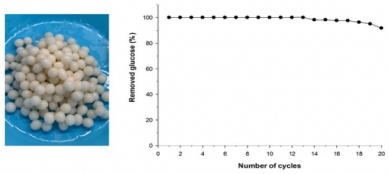
For second step we searched a microorganism that was capable of consuming D-glucose without consuming D-galactose from the hydrolyzed whey permeate; and the winner was the crabtree negative microorganism Komagataella phaffii (Pichia pastoris). Thus, 350 mg of wet weight cells were immobilized in alginate gel beads, and the resulting biocatalyst was able of completely eliminating D-glucose and efficiently reused for 13 cycles [3].

The third step was the isomerization of D-galactose with a L-arabinose isomerase from Bacillus stearothermophilus. The enzyme was successfully immobilized onto an amino polymethacrylate support and a packed bed bioreactor was developed and operated continuously for 9 days at 50 °C, with an initial productivity of D-tagatose of 77 g L-1 day-1.

The fourth step was the search of a microorganism able to consume D-galactose without consuming D-tagatose. From 19 microorganisms tested, the best was Schwanniomyces occidentalis. This microorganism was immobilized with alginate and it could be reused for 3 cycles of 5 h each.

As a conclusion, we have demonstrated that it is possible to obtain D-tagatose using an industrial waste as starting reagent, following a completely biocatalytic methodology.

FIGURES



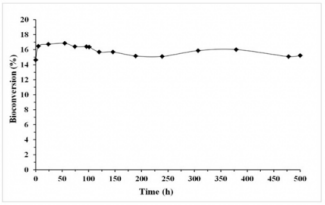


FIGURE 1

cycles with P. pastoris

Percentage of eliminated D-glucose in different cycles with P. pastoris immobilized in calcium alginate beads, using a mixture of D-galactose and D-glucose (50 mg/mL of each sugar) as substrate.

FIGURE 2

Bioreactor of L-arabinose isomerase

Continuous packed bed reactor of L-AI US100 immobilized on Sepabeads EC-EA at 50 °C during 9 days of operation. The flow of the feeding solution (90 g/L galactose, 0.5 mM MnSO4 in 0.1 M MOPS buffer pH 7.5) was maintained at 0.03 mL/min.

KEYWORDS

D-tagatose | Biocatalysis | immobilization | bioreactors

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