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Comparative study on different strategies involved for xylitol purification from culture media fermented by *Candida tropicalis*

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ABSTRACT

Xylitol, a sugar substitute, is a high value product for pharmaceutical and food industries and its purification being of commercial importance. In the present study, the purification of xylitol obtained through *Candida tropicalis* by fermentation using synthetic xylose and corn cob hemicellulosic hydrolysate as substrates were studied for liquid–liquid extraction (21.72 g/l xylitol extracted in 1:5 (v/v) of ethyl acetate) and precipitation (67.44% xylitol recovery along with certain impurities). By this method xylitol recovery is difficult and expensive for large scale processes. Therefore, activated charcoal treatment followed by vaccum concentration and crystallization method for xylitol extraction was evaluated. The optimized conditions obtained for activated charcoal treatment followed by vaccum concentration and crystallization method were 15.0 g/l of charcoal concentration at 30 °C for 1 h with 10 times super saturation of initial concentration and crystallization temperature of −20 °C for initiation and then at 8 °C yielding 43.97%. After 4 cycles of crystallization, 76.20% and 68.06% xylitol crystallization yield was obtained in 50 ml and 5.0 l of the synthetic xylose fermentation broth by adapted strain of *C. tropicalis* respectively. The effect of solvents on the crystalline structure of xylitol showed prismatic structure in the presence of ethanol and orthorhombic needles in the presence of tetrahydrofuran. The purity of the xylitol was characterized using ^{13}C and ^{1}H nuclear magnetic resonance, mass spectroscopy, and optical rotation, confirming 98.99% purity in a pure crystallized form.

1. Introduction

Industries producing polyol sweeteners have registered a growing demand for the consumption of sugar-free and low heat value products. Among these, xylitol is an important sugar substitute with certain interesting physical and chemical properties which make it a high value compound for pharmaceutical, odontological and food industries. At present, large scale commercial production of xylitol is by an expensive catalytic hydrogenation of D-xylose from acid hydrolysis of lignocellulosics [1]. Hence, it is worthwhile to explore an alternative process for the effective production of xylitol using micro-organisms which make use of the semi synthetic media [2,3] or detoxified hemicellulosic hydrolysate [4] in order to reduce the manufacturing costs with minimal environmental and energy issues [5].

The recovery and purification of the product exists as a very complicated step in several industrial fermentative processes, which majorly depend on the nature of the product as well as on the complex composition of the fermentation broth [6]. In order to recover a product which requires higher purity for commercialization [7], it is often implied that important steps characterized by costs even higher than the production process are used. However, in literature on polyols, very little information is available about xylitol recovery [8–10] and mainly reports are related to the obtainment and treatment of the hemicellulosic hydrolysate, its fermentation and metabolic bioconversion [11,12]. Until now, on industrial scale, the xylitol obtained is separated and purified by chromatographic methods [13,14]. Jandera and Churacek [15] used cation exchange resin columns for xylitol separations followed by crystallization at low temperatures of the xylitol-rich solutions. Whereas, Gurgel et al. [8] used both anion and cation exchange resins to purify xylitol from sugarcane bagasse hydrolysate fermentation broth and observed a xylitol loss of about 46–57%. However, such techniques tend to be expensive for industrial scale processes.

In order to overcome this hurdle, an efficient and economically competitive strategy for xylitol purification and recovery from fermented broth was developed. The purification of solutions by liquid–liquid extraction and precipitation is used in numerous industrial processes in order to recover dissolved substances or to remove undesirable impurities. However, the most efficient strategy used for xylitol purification and its extraction is the activated charcoal treatment followed by vaccum concentration and crystal-

Abbreviations: NMR, nuclear magnetic resonance; OR, optical rotation; MS, mass spectroscopy; HPLC, high performance liquid chromatography.

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lization method [16]. In accordance with the literature reports, it is well known that in the past few years, xylitol crystallization has drawn more attention [17] and is believed to be the final step for obtaining highly purified products [18,19]. The choice of the crystallization method (cooling, evaporation, precipitation and salting out) is dependent on the solubility and saturation slopes with the temperature [20]. Mullin [21] explained the three zones involved in the process of crystallization wherein he stated that at a very low saturation concentration there was no possibility of nucleation or crystal growth. The existing crystals dissolve in the medium. Secondly, in supersaturated metastable zone, there was an occurrence of crystal growth but spontaneous nucleation does not take place. It is believed to be the first stage in the crystallization process or also known as primary nucleation wherein a series of bimolecular collisions occurs and forms an aggregate of small number of molecules of dissolved materials (embryos). Thirdly, there is a labile zone where nuclei are formed spontaneously from a clear solution. However, according to Martínez et al. [20], these three zones are controlled not only by equilibrium, but also by process parameters like agitation, temperature, solution purity and cooling rate. Martínez et al. [4] also reported that during crystallization at lower temperatures thermal degradation of compounds sensitive to heat is minimized and also its unit operating costs exist lowered as compared to other recovery techniques due to high product concentration.

The aim of the present investigation is to compare various purification strategies for efficient xylitol purification and its extraction from synthetic as well as detoxified corncob hemicellulosic hydrolysate fermented by a natural yeast isolate of *C. tropicalis* which proved to be an efficient xylitol producer. The purification strategy selected was optimized for maximum xylitol crystallization yield and to evaluate its process economics.

2. Experimental

2.1. Chemicals used

The xylose used as substrate was obtained from corncob through acid hydrolysis. The hydrolysate was detoxified using a combination of activated charcoal and pH adjustment followed by 2-fold concentration in rotavapor. The initial concentration obtained through this process is 40.16 g/l of xylose (details not given). The synthetic xylose was purchased from Central Drug House (CDH, Mumbai, India). All other medium components and chemicals used were of analytical grade and were purchased locally (Himedia, Qualigenes and Sisco Research Laboratories Ltd., India).

2.2. Media and fermentation conditions

The fermentation broth was prepared using synthetic xylose (100.0 g/l for (unadapted) parent strain and 175.0 g/l for adapted strain of *Candida tropicalis*) and corncob hemicellulosic hydrolysate (obtained by acid hydrolysis) containing 40.16 g/l of xylose. The medium was supplemented with (in g/l) yeast extract 5.0, KH_2PO_4 2.0, and $MgSO_4 \cdot 7H_2O$ 0.3. The appropriate medium was inoculated with 5.0% of the seed inoculum and fermentation was carried out at 30 °C in 10 l fermentor with 5 l working volume (New Brunswick Sci. Inc. Fermentor Bioflow IV, USA). The pH was controlled automatically with 1 N NaOH/1 N HCl using a pH controller at pH 4.5. Agitation and aeration rate were adjusted to 400 rpm with a constant rate of 0.7 vvm (up to 24 h) and then shifted to 200 rpm and 0.3 vvm for rest of the fermentation run. Foaming was controlled by adding silicon antifoam agent (50%, v/v, prepared in distilled water). Samples were withdrawn periodically at intervals of 6 h and analysed for xylitol production. The fermentation run was stopped after 60 h (unadapted strain) and 42 h (adapted strain) in corncob hemicellulosic hydrolysate medium and 84 h (unadapted strain) and 96 h (adapted strain) of incubation in the presence of synthetic xylose by this time more than 90–95% xylose was consumed. The fermented broth was centrifuged at 10,000 rpm for 15 min in order to separate the cells and solid particles (Sigma centrifuge 4X15, Germany).

2.3. Extraction of xylitol from fermented broth

Three different methods were investigated, analysed and compared for xylitol purification from culture broth.

2.3.1. Liquid–liquid extraction

The filtered fermented broth (50 ml) was extracted with ethyl acetate, chloroform or dichloromethane in the ratio of 1:1 (v/v) at 30 °C. The aqueous and organic fraction obtained with these solvents was analysed for xylitol using HPLC.

2.3.2. Precipitation

The filtered fermented broth was mixed with each of ethanol, acetone or tetrahydrofuran (THF) in 1:1 ratio (v/v), stirred and allowed to stand for 60 min at 4 °C. The precipitate was separated by centrifugation (Sigma centrifuge 4X15, Germany) at 4000 rpm for 10 min. The precipitate thus obtained was dissolved in water and the left over solution was analysed for xylitol using HPLC.

2.3.3. Vaccum concentration and crystallization

2.3.3.1. Treatment with activated charcoal. After micro filtration (GE Healthcare, USA), aliquots of the filtrate (50 ml) were transferred into 250 ml flasks for the treatment with 20 g/l of charcoal concentration at 30 °C. After magnetic agitation for 1 h, the mixture was filtered using Whatmann filter paper No. 1. Samples of the filtrate were analysed for the determinations of the initial concentrations of xylitol.

2.3.3.2. Crystallization tests. After treatment with activated charcoal, fermented broth was concentrated in rotavapor (Buchi Rotavapor R-210, Germany) at 55 ± 5 °C up to the achievement of the selected concentration. The aliquots of the concentrated solutions were transferred to the glass petri plates and to that finely ground commercial xylitol (1.0 g/l) was added in order to favor nucleation of the crystals. The petriplates were kept at −20 °C in order to initiate crystal formation and once the process initiates the petriplates were shifted at 8 °C to increase the crystallization yield.

2.4. Precipitated crystals estimation

After completion of crystallization, precipitated crystals were removed from mother liquor through filtration using Whatmann filter paper No. 1 and dried on the filter paper at room temperature. Preweighted crystals were dissolved in water to determine their contents of xylitol and its purity using HPLC.

2.5. Process optimization to enhance crystallization

Effect of various parameters such as treatment temperature (20–50 °C), different concentrations of activated charcoal (5.0–25.0 g/l), contact time (15–60 min.), xylitol saturation concentration (5–15 times), crystallization temperature (−20 °C, 8 °C and −20 °C for 3–4 days and then transferred to 8 °C), presence of xylose in the concentrated broth were evaluated using one variable at a time approach in order to enhance the crystallization yield and the quality of crystals formed.