Improvement in xylitol production from wheat straw hemicellulosic hydrolysate achieved by the use of immobilized *Aspergillus terreus* cells

Alka Tangri, Ruchi Bajpai and Lalit K. Singh

Abstract

Aspergillus terreus cells were immobilized in Ca-alginate beads and used for xylitol production from concentrated wheat straw hydrolysate during successive fermentation batches, each lasting 48 h. The maximum yield ($Y_{g/g}$) and volumetric productivity (Q_p, g/L.h) of xylitol by the immobilized *Aspergillus terreus* cells were calculated. Although the results were very close but the inoculum to polymer ratio of 1: 2 showed better results with 0.84 g/g yield and 1.74 g/L.h volumetric productivity than the other ratios tested. Thus 33% v/v *Aspergillus terreus* mycelia inoculum with respect to 3% sodium alginate was found suitable for immobilization of *Aspergillus* cells with the ultimate aim of xylitol production from wheat straw hemicellulose hydrolysate.

Key words ; *Aspergillus terreus*, Ca-alginate, immobilization, wheat straw.

1. INTRODUCTION

XYLITOL is a polyalcohol with sweetening power similar to sucrose and important industrial applications mainly because of its anticariogenic properties. Currently, pure xylose obtained from hemicellulosic hydrolysates is used as a substrate to produce xylitol from high pressure and temperature chemical process.

The fermentative process can be an interesting approach to the xylitol production due to the use of mild condition of pressure and temperature, in addition to the possibility of using the hemicellulosic hydrolysate without the previous xylose purification. Aiming to improve the performance of this bioprocess research about the use of immobilization methods have been developed. Its recommendable to use cell immobilization not only to enhance cell stability, but also to maximize the fermented broth fraction discarded at the end of each batch, facilitate the reutilization of the biocatalysts and minimize time and separation costs. One of the most effective immobilization methods is gel entrapment, Ca-alginate being the commonest support used for the immobilization of viable cells, since it does not require drastic conditions.

There are many advantages of using immobilized cells systems, as the facility of their separation from the medium because of its distinctive characteristics related with the liquid medium.

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Because of this facility, the use of immobilized cells in semi continuous mode operation is indicated, maintaining the cells into reactor along the batches. The inoculum step can be eliminated and the cells can be adapted to the culture medium, resulting in higher productivities and yields. Wheat straw is one of the most abundant by products generated in India because of its large production. This biomass has a high sugar content of sugars and can be converted into valuable products, including xylitol [1]. Xylitol is a polyol, discovered in 1891, that has sweetening power similar to sucrose, but it present some advantages with respect to sucrose, such anticariogenicity, negative heat of dissolution, absence of the Maillard reaction, insulin-independent metabolism, higher chemical stability, and several biomedical properties[1, 2]. It is an edulcorant recognized since the1960s and is commonly used for oral hygiene as well as the preparation of chewing gum, comfits, and pharmaceutical and cosmetic products.

Xylose, which is present in the hemicellulosic fraction of wheat straw, can be converted into xylitol by chemical catalysis [3] or microbial bioconversion [4]. Some microorganisms, especially xylose fermenting yeasts, can decrease xylose to xylitol by action of Nicotinamide Adenine Dinucleotide Phosphate (NADPH)- and/or NADH-dependent xylose reductase (XR). Xylitol is oxidized to xylulose by NAD+-dependent xylitol dehydrogenase (XDH), and then xylulose is phosphorylated by xylulosekinase to xylose-5-phosphate, which can enter the pentose phosphate and, subsequently, the glycolytic pathway [5]. Several researchers have explored the microbial process of xylitol production in past years because wheat straw biotechnologic utilization seems to be a promising alternative when compared with

chemical synthesis [6,7]. The chemical process does in fact require high temperature and pressure [4], and xylose purification before chemical reaction [therefore, the resulting product Seeking to develop a bioprocess for xylitol production from wheat straw, several studies concerning xylose-to-xylitol bioconversion in immobilized cells systems have been previously developed [8–10]. The use of immobilized cell preparations in biotechnological processes allows easier development of continuous process, higher productivity, easier product recovery and refinement, repeated use of the entrapped cells in successive cycles [11], and protection of the entrapped biocatalysts against adverse environmental conditions [12, 13]. Previous studies also demonstrated that repeated-batch operation with cell recycling can be an interesting alternative to improve the performance of xylose-to-xylitol bioconversion because it promotes cell adaptation to the medium and, as a consequence, increases the substrate-to-product conversion rate with multiple batches [14, 15]. Several supports for cell immobilization have been successfully tested for xylitol production, such as Ca-alginate [8–10], porous glass, zeolite [14], sugarcane bagasse [16], and polyvinylalcohol (PVA) gel [12]. PVA is inexpensive, easily available, and nontoxic to microorganisms, synthetic polymer, which is widely used as a biomaterial, in the textile industry, and more recently as a carrier for cell and enzyme immobilization [17, 18]. The aim of this study was to evaluate the performance and stability of xylitol production from wheat straw hydrolysate entrapped *Aspergillus terreus* cells in-batches with cell recycling.

2. MATERIALS AND METHODS

2.1 Preparation of Hemicellulose Hydrolysate

Hemicellulose hydrolysate was prepared by treating the wheat straw as substrate with acid and heat. 6 ml of 2% H_2SO_4 was added to 300 mg of each substrate. The mixture was then allowed to stand for 1 h at30ºC. 168 ml of distilled water was then added and mixture was autoclaved at 125ºC for 1 h. Pre- hydrolysate was filtered to remove the unhydrolyzed and washed with warm water (60ºC).

The filtrate and washings were pooled together and made up to 1 l. The filtrate was then concentrated three to four folds by evaporation under vacuum at 60±5ºC to increase xylose concentration in the hydrolysate and stored at 4ºC for further use.

2.2 Microorganism and Inoculum Preparation

Growing mycelia of *Aspergillus terreus* were used for immobilization. *Aspergillus terreus* cells were grown at 30°C for 36 h. 150 ml culture broths were harvested by centrifuge at 5000 rpm for 10 min. The fungal strain used in this study was isolated from soil and identified as *Aspergillus terreus var. africana*. The strain was maintained on MXYP agar medium (malt extract: 3 gL^{-1}; xylose: 10gL^{-1} yeast extract: 3 gL^{-1}; peptone: 5 gL^{-1}; agar: 15gL^{-1}). The pH of the medium was kept at 7.0 and was sterilized at 121°C (15 psi) for 20 min. The culture was incubated at 30°C and 150 rpm for 24 h. One loopful of culture was then transferred into test tubes containing 10 ml of liquid medium of above composition and incubated at same conditions. Culture was stored on agar slants at 4°C till further use and was sub cultured after every 28 days.

2.3 Cell Immobilization

Growing mycelia of *Aspergillus terreus* were used for immobilization. *Aspergillus terreus* cells were grown at 30°C for 36 h. 150 ml culture broths were harvested by centrifuge at 5000 rpm for 10 min. In aseptic conditions the seeding mycelial pellets of fungus were immobilized in Ca-alginate. In general, 50 ml of this growth medium was mixed with an equal volume (1:1 v/v) of 4% (w/v) Na-alginate solution. A 100 ml aliquot of alginate-cell suspension containing 2% Na-alginate was added drop wise to 1000 ml of 2% $CaCl_2$ with a syringe. Alginate drops solidified upon contact with $CaCl_2$, forming beads and thus entrapping fungal mycelia. The size of spherical beads was 2.5±0.3 mm. The spherical beads were allowed to harden for 30 min and then were washed with sterile saline solution (0.85% NaCl) to remove excess calcium ions and mycelia and stored at 4ºC for 24 h. The detoxified wheat straw hydrolysate was supplemented with the same nutrients as described earlier. Erlenmeyer flasks (250 ml) containing 100 ml fermentation medium and 25 g beads and kept in a rotary shaker at 150 rpm at 30°C for 96.

2.4Analytical Methods

Dry cell weight was estimated using a calibration curve made from the relationship between optical density at 540 nm and dry cell weight. Cellulose content was estimated using Semi-micro determination Method of Updegraff [19]. Pentose sugar analysis was carried out by a modification of the method initially proposed by Roe and Rice [20]. Modified Klason Lignin method ASTM D-1106 was used for determination of lignin content. Ash content was determined using ASTM D2584, D5630, ISO 3451 Accredited method. The concentration of xylitol was determined by high performance liquid chromatography (HPLC, Waters Corporation, Milford Massachusetts, USA) with BioRad Aminex HPX-87H column, at 45°C, with 0.01N H_2SO_4 as the mobile phase, at a flow rate of 0.6 ml/min and a refractive index (RI) detector. Immobilized cells concentration was estimated by the difference between the

dry weights of the porous glass particles before and after fermentations.

3. RESULTS AND DISCUSSION

3.1 Effect of inoculum to polymer ratio on the performance of immobilized Aspergillus terreus

To study the effect of *Aspergillus terreus* mycelia inoculum concentration with respect to the concentration of sodium alginate used as polymer for immobilization, three different ratios were selected; 1:1, 1:2 and 1:3 corresponding to 50%, 33% and 25% v/v inoculum concentration. Concentrations of sodium alginate and calcium chloride were kept at 2% w/v each. Three different sets of experiments were conducted using same amount of immobilized *Aspergillus terreus* mycelia beads. Samples were withdrawn at every 24 h and analyzed for xylitol production.

The maximum yield ($Y_{g/g}$) and volumetric productivity (Q_P, g/L.h) of xylitol by the immobilized *Aspergillus terreus* cells is shown in Figure 1. Although the results were very close but the inoculum to polymer ratio of 1: 2 showed better results with 0.84 g/g yield and 1.74 g/L.h volumetric productivity than the other ratios tested. Thus 33% v/v *Aspergillus terreus* mycelia inoculum with respect to 3% sodium alginate was found suitable for immobilization of *Aspergillus* cells with the ultimate aim of xylitol production from wheat straw hemicellulose hydrolysate

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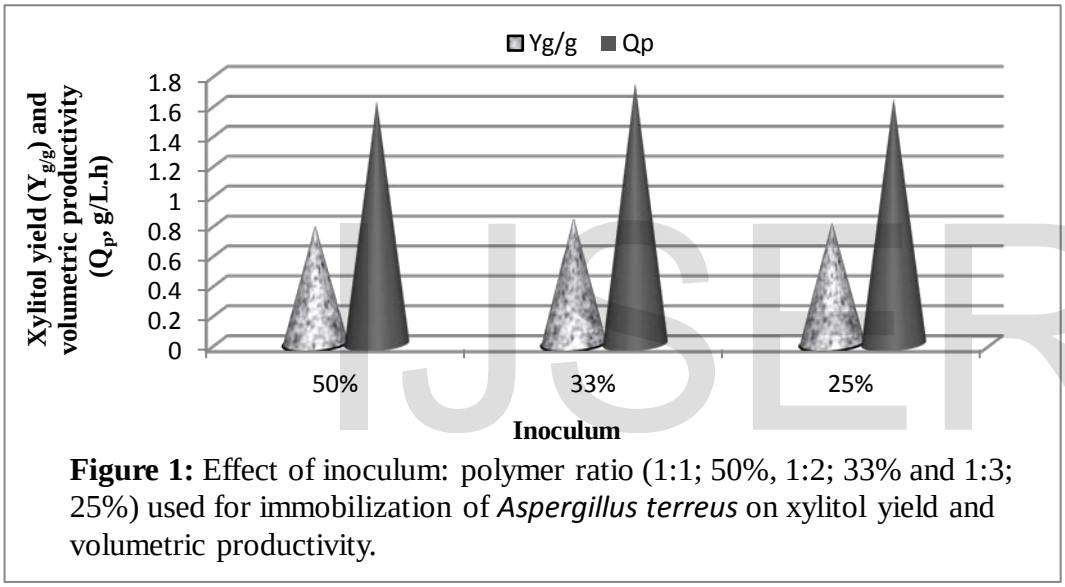

Figure 1: Effect of inoculum: polymer ratio (1:1; 50%, 1:2; 33% and 1:3; 25%) used for immobilization of *Aspergillus terreus* on xylitol yield and volumetric productivity.

3.2 Effect of concentration of polymer (Na-alginate) on the performance of immobilized Aspergillus terreus

Different concentrations of polymer (Na-alginate) were tested to study the effect of polymer concentration used for immobilization of *Aspergillus terreus* mycelia for xylitol production. 2%, 3% and 4% Na-alginate was used to prepare spherical *Aspergillus terreus* mycelial beads. Three different sets of experiments were conducted using same amount of immobilized *Aspergillus terreus* mycelia beads. Samples were withdrawn at every 24 h and analyzed for xylitol production.

The maximum yield ($Y_{g/g}$) and volumetric productivity (Q_P, g/L.h) of xylitol by the immobilized *Aspergillus terreus* cells is shown in Figure 2. Although the results were very close but the highest value of yield ($Y_{g/g}$) and volumetric productivity (Q_P, g/L.h) of xylitol were found as 0.84 g/g and 1.75 g/L.h corresponding to 3% w/v polymer concentration. Thus 3% w/v Na-alginate as polymer for preparing immobilized *Aspergillus terreus* mycelia beads with the ultimate aim of xylitol production from wheat straw hemicellulose hydrolysate.

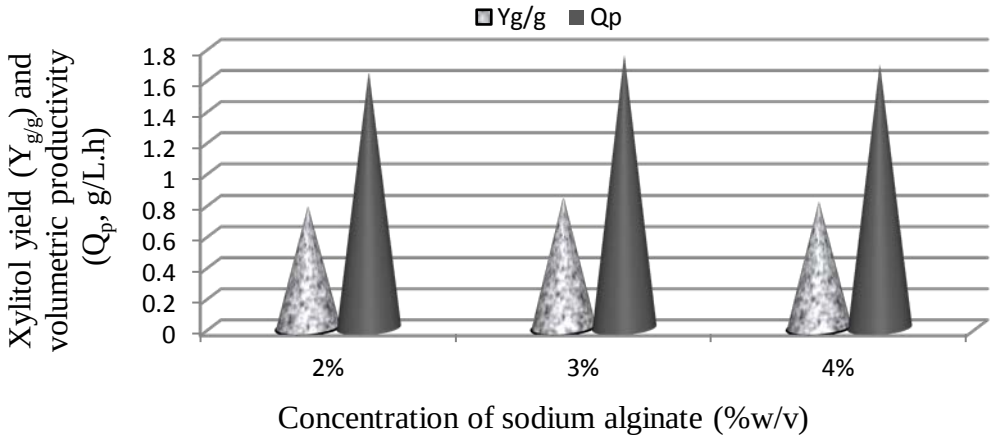

Figure 2: Effect of concentration of polymer (sodium alginate) used for immobilization of *Aspergillus terreus* on xylitol yield and volumetric productivity.

3.3 Effect of concentration of gel inducer ($CaCl_2$) on the performance of immobilized Aspergillus terreus

Calcium chloride was used as gel inducer for preparing immobilized *Aspergillus terreus* beads. Different concentrations of calcium chloride were used to identify its effect on the quality of beads to be used for xylitol production. 1%, 2% and 3% calcium chloride was used to prepare spherical *Aspergillus terreus* mycelial beads. Three different sets of experiments were conducted using same amount of immobilized *Aspergillus terreus* mycelia beads. Samples were withdrawn at every 24 h and analyzed for xylitol production.

The maximum yield ($Y_{g/g}$) and volumetric productivity (Q_P, g/L.h) of xylitol by the immobilized *Aspergillus terreus* cells using different concentrations of calcium chloride is shown in Figure 3. Although the results were very close but the highest value of yield ($Y_{g/g}$) and volumetric productivity (Q_P, g/L.h) of xylitol were found as 0.83 g/g and 1.72 g/L.h corresponding to 2% w/v gel inducer (calcium chloride) concentration. Thus 2% w/v $CaCl_2$ as gel inducer for preparing immobilized *Aspergillus terreus* mycelia beads with the ultimate aim of xylitol production from wheat straw hemicellulose hydrolysate.

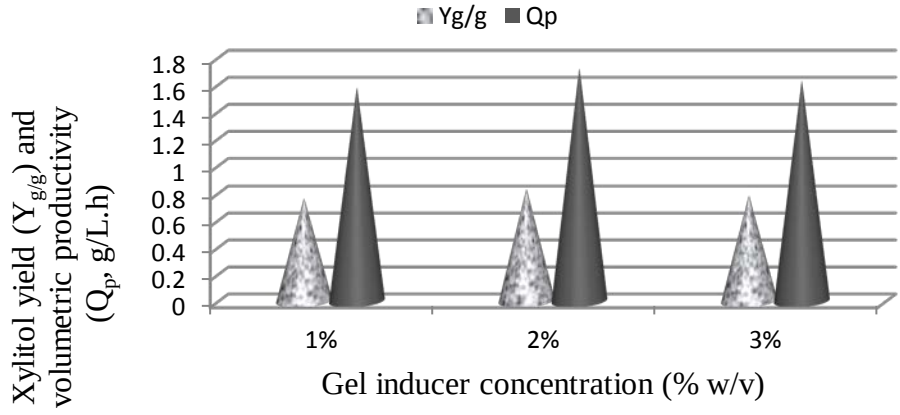

Figure 3: Effect of concentration of Gel inducer (calcium chloride) used for immobilization of *Aspergillus terreus* on xylitol yield and volumetric productivity.

4. CONCLUSION

Owing to its properties xylitol is being extensively used in food, odontological and pharmaceutical industry

Study of this research work suggested that economical production of xylitol could be achieved by using immobilized *Aspergillus terreus*. In this regard wheat straw hemicellulose hydrolysate was utilized as raw material for xylitol production with high hemicellulosic fraction (28.3%w/w) and low lignin content (16.6% w/w) as compared to other raw materials. Immobilized cell technology posses several advantages like use of immobilized cells provided opportunity of reuse, potential to increase the xylitol yield by eliminating unit process used. Thus, process expenses in cell recovery and reutilization can be minimized.

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