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# Polyvinyl Alcohol Beads as a Robust and Reusable Carrier for *Pichia fermentans* WC1507 in Xylose-to-Xylitol Bioprocessing

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The bioconversion of xylose into xylitol offers a sustainable approach to upcycle pentoses in lignocellulosic hydrolysates by leveraging the metabolic capabilities of ascomycetous yeasts. Scalable solutions, such as immobilized-cell systems, facilitate industrial bioprocessing by enabling easy biomass recovery, reusability, and maintenance of high cell concentrations without compromising productivity. *Pichia fermentans* WC 1507 has demonstrated remarkable efficiency in converting xylose to xylitol. In this study, its cells were immobilized in beads composed of 0.8% (w/v) alginate and 10% (w/v) polyvinyl alcohol (PVA) crosslinked with calcium and boric acid. While immobilization initially reduce yeast viability, it was progressively restored during the first utilization cycles of the PVA-immobilized cells.
The PVA beads loaded with the cells were tested in four consecutive 7-day fermentation runs using a medium containing 120 g/L xylose. The experiments confirmed the excellent robustness of the PVA beads, that proved to be usable in stirred tank bioreactors for remarkably long periods. Across the four runs, fermentation efficiency improved, achieving stable xylitol yields of 60–63 g/L, with 54–56% conversion and a volumetric productivity of 450–470 mg/L/h under non-optimized conditions.
These results highlighted the potential of *P. fermentans* WC 1507 for the valorization of xylose-rich hydrolysate and validated PVA matrices as durable biomass carrier for use in stirred bioreactor systems.

## 1. Introduction

Sugar alcohols, such as xylitol and arabitol, aren ranked among the top 12 value added chemicals derivable from biomasses (Werpy et al., 2004; Raimondi et al., 2022). Xylitol, in particular, is a five-carbon sugar alcohol with diabetic-friendly sweetening power, anti-cariogenic properties, and the ability to inhibit microbial growth (Morais Junior et al., 2019). It finds extensive application in food, dental, and pharmaceutical products, with a global market demand of 200 billion tons per year, projected to grow from 2021 to 2026 (Ravella et al., 2022).
The bioconversion of xylose into xylitol is a valuable strategy for upcycling the pentoses contained in lignocellulosic hydrolysates. This transformation can be achieved through the metabolic capability of certain ascomycetous yeasts, and several fermentation processes has been proposed so far. Notably, the most extensively studied yeasts for this purpose belong to the genera *Debaryomyces* (e.g., *D. nepalensis*, *D. hansenii*), *Candida* (e.g., *C. intermedia*, *C. tropicalis*, *C. boidinii*, *C. parapsilosis*), and *Meyerozyma* (*M. guilliermondii* and *M. caribbica*) as well as species like *Barnettozyma populi*, *Scheffersomyces stipites* and *Kluyveromyces marxianus* (Prakash et al., 2011; Wu et al., 2018; Tamburini et al., 2015; Saha and Kennedy, 2020; Queiroz et al., 2022; Kumar et al., 2022). *Pichia fermentans* deserves to be added to these species (Prabhu et al., 2020). Specifically, the strain *P. fermentans* WC 1507 was demonstrated to be remarkably efficient in converting xylose to xylitol, achieving promising yields and titres (Raimondi et al., 2023; Ranieri et al., 2024a).
Adopting biotechnological methods for producing value-added products requires scalable solutions for industrial processes. Immobilized-cell systems offer promising features for bioprocess scale-up, including easy biomass recovery, reusability across fermentation batches, and the ability to maintain high cell concentrations without

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compromising the biocatalyst's efficiency. Consequently, there is a formidable scientific as well as industrial interest for applications of immobilized cells (biocatalysts) in various fermentation processes (Nunes et al., 2016; Zhang and Ye, 2014). However, cells immobilization poses several challenges including reduced cell viability due to immobilization conditions, mass transfer limitations (particularly for oxygen, substrates, and products), and various technological problems (Willaert, 2011). These include poor mechanical properties of immobilization matrices, such as softness or swelling, which can complicate their use in certain bioreactor configurations (Ranieri et al., 2024b).
Polyvinyl alcohol (PVA) is a semi-crystalline nontoxic synthetic polymer with extraordinary biocompatibility, chemical resistance and mechanical properties (Mohd Zain et al., 2010; Amri et al., 2016). Its diverse applications includeadhesion products, food packaging, tissue scaffolding, drug release wound dressings, filtration materials and membranes (Guzman-Puyol et al., 2015). In recent years, PVA has been widely investigated for cell immobilization, as cross-linking its hydroxyl groups with agents such as boric acid forms a durable reticulum with outstanding mechanical strength (Candry et al., 2022).
*P. fermentans* WC 1507 was previously demonstrated to be remarkably efficient in transforming xylose to xylitol. Cells of this strain immobilized within calcium alginate beads were also utilized with some success in preliminary fermentation processes that, however, resented mass transfer limitations since the softness calcium alginate made the beads unusable in mechanically stirred bioreactors (Ranieri et al., 2024b). In the present study, the cells of *P. fermentans* WC 1507 were immobilized in PVA beads, with the aim to evaluate whether the process with immobilized cells could be applied in a stirred tank bioreactor.

## 2. Materials and methods

### 2.1 Strain, media, and culture conditions

The strain *P. fermentans* WC 1507, belonging the collection of the Laboratory of Microbial Biotechnologies (Department of Life Sciences, University of Modena and Reggio Emilia), was routinely cultured in YPD (20 g/L glucose, 10 g/L tryptone, 10 g/L yeast extract) broth at 30°C in aerobic conditions.
The biomass for cells immobilization was prepared in MY medium (20 g/L xylose, 3 g/L yeast extract, 2 g/L $(NH_4)_2SO_4$, 3 g/L $KH_2PO_4$, 1 g/L $K_2HPO_4$, and 1 g/L $MgSO_4 \times 7H_2O$), where the yeast was incubated aerobically at 30 °C. Transformation of xylose into xylitol was carried out in MY medium containing 120 g/L. All the chemicals were obtained from Sigma-Aldrich (Steinheim, Germany) unless otherwise stated.

### 2.2 Cells immobilization

For immobilization in PVA beads, the biomass of *P. fermentans* WC 1507 was grown in MY medium with 20 g/L xylose for in 24 h. The cells were harvested by centrifugation (4000 × g for 5 min at 4°C), washed twice with saline solution (9 g/L NaCl), and resuspended 10-fold concentrated in the saline solution. One volume of cells suspension was mixed to five volumes of a solution containing 12% (w/v) PVA (MW = 72 KDa, DH = 97.5–99.5%) and 9.6% (w/v) sodium alginate. The suspension was thoroughly mixed and pumped through a syringe needle into a gently stirred solution 20 g/L $CaCl_2$ and 50 g/L boric acid. After 30 min of incubation at room temperature, the beads were collected, rinsed with saline solution, and submerged in gently stirred 0.5 M $Na_2SO_4$ for 1.5 h.
As indicated for specific experiments, the beads were utilized directly in fermentation experiments or were rinsed and submerged for 15 min at room temperature in 9 g/L NaCl or in 150 g/L glycerol.
All the steps of beads preparation were carried out under aseptic conditions, utilizing autoclaved solutions and materials.

### 2.3 Xylose to xylitol transformation

PVA beads loaded with *P. fermentans* WC 1507 cells were utilized as biocatalyst in the conversion of xylose into xylitol in flasks and bioreactor experiments. All the fermentations were carried out with a beads:medium ratio of 1:10 (w/v), utilizing MY medium containing 120 g/L xylose.
Flasks experiments were carried out in 250 mL flasks containing 50 mL of medium and 5 g of PVA beads. The flasks were incubated at 200 rpm in an orbital shaker at 30°C.
Bioreactor experiments were carried out in 500 mL stirrer tank bioreactors (Mini Bio, Applikon Biotechnology, Delft, the Netherlands) containing 350 mL of medium and 35 mL of beads. The bioreactors were thermostated at 30 °C and stirred at 800 rpm. The pH was continuously measured and automatically titrated at the value of 3.5. No automatic control of stirring was applied to prevent the decrease of dissolved oxygen tension (DOT). All the processes were periodically sampled to determine xylose consumption, and xylitol generation. To evaluate the reusability of PVA beads across consecutive fermentation runs, exhaust broth was removed from the bioreactor and replaced with fresh MY medium containing 120 g/L xylose.
The xylose to xylitol conversion yield ($Y_{P/S}$) was calculated on mass basis as the ratio between xylitol produced

and xylose consumed. The Volumetric productivity ($Q_P$) was calculated by dividing xylitol concentration by time.

### 2.4 Chemical analysis

Xylose and xylitol were quantified by HPLC with refractive index detector (1200 System, Agilent Technologies, Waldbronn, Germany) and Aminex HPX-87 H ion exclusion column. Isocratic elution was carried out at 60°C with 0.6 ml $min^{-1}$ of 5 mM $H_2SO_4$ (Raimondi et al., 2022). The analytes were identified by comparison of the retention time with that of a standard solution.

### 2.5 Statistical analysis

All values are means of three separate experiments. *t*-test and ANOVA followed by Tukey *post hoc* comparisons were utilized for the comparison of means. Differences were considered statistically significant for $p < 0.05$.

## 3. Results and discussion

In the present study, it was investigated whether cells of *P. fermentans* WC 1507, already known for its xylose-to-xylitol conversion ability, could be entrapped within PVA beads to be utilized in a stirred tank bioreactor for the production of xylitol. The beads were loaded with $2.5 \times 10^8$ cells of *P. fermentans* WC 1507 per g (wet weight) and utilized in fermentation experiments in flasks and in bioreactors.

### 3.1 Beads preparation and evaluation in shake flasks

PVA crosslinked with boric acid is a cost-effective material with a rubber-like elasticity and good mechanical properties. It has been increasingly utilized for the immobilization of microbial cells within highly durable, high-strength beads, making it particularly useful in fermentative processes. The main drawbacks of this immobilization technique reside in the toxicity of boric acid, that compromise yeast viability, and in the occurrence of agglomeration and swelling, that are generally encountered during beads preparation (Takei et al., 2012; Dinh and Bach, 2014). The agglomeration and swelling problems were effectively eliminated by including a small amount of calcium alginate. With regards crosslinking toxicity, a preliminary experiment revealed that 1 h contact of free cells of *P. fermentans* WC 1507 with boric acid had the effect of reducing yeast viability by approx. 1 magnitude order. Therefore, after 30 min incubation in boric acid, the beads were washed and soaked in $Na_2SO_4$, with the aim of both removing the excess of boric acid and replacing it with sulfate in the PVA reticulum (Dinh and Bach, 2014).

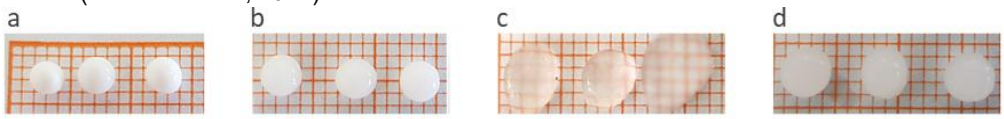


*Figure 1: PVA beads loaded with P. fermentans WC 1507 cells, photographed on millimiter-grid paper after the treatment with boric acid (a), after soaking in $Na_2SO_4$ (b) and after washing with saline (c) or glycerol (d) solutions.*

The obtained PVA beads were opaque and solid, with a diameter of approx. 3 mm after boric acid treatment and ranged between 3 and 4 mm after soaking in $Na_2SO_4$ (Fig. 1a and 1b). The beads were then added to flasks of MY medium containing 120 g/L xylose to evaluate xylose production. After 216 h of incubation, a minor amount of xylose was consumed (14.5 g/L) and only 3.5 g/L xylitol was generated (Fig 2A). These values were remarkably lower than those obtained in first cycle of utilization of alginate beads loaded with the same strain, that consumed 35 g/L xylose and generated 10 g/L xylitol in 116 h (Ranieri et al., 2024a). This reduction was attributed to a loss of viability in *Pichia fermentans* WC 1507 during the immobilization process, likely due to exposure to boric acid, as previously reported for other yeast strains (Takei et al., 2011; Nunes et al., 2016). However, when the medium was replaced, the conversion proceeded more rapidly compared with the first run, suggesting that the yeasts cells had progressively recovered in viable numbers (Fig 2A).
To mitigate viability loss and increase the efficiency, the beads were thoroughly washed and soaked in sterile saline solution to remove excess of the crosslinking agents. As a result, the beads were jelly, translucent and lightly swollen, with diameter in the range of 4–6 mm (fig 1c). Concurrently, transformation efficiency was significantly increased in the first run ($p < 0.05$), that led to the consumption of 91 g/L xylose and to the generation of 57 g/L xylitol (Fig 2B). Similar values were obtained also during the second run, after the spent medium was replaced with fresh one.

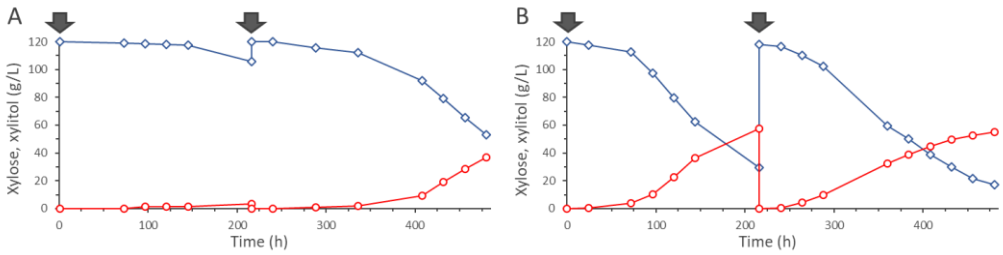


*Figure 2: Time-course of shake flasks fermentation runs with PVA beads loaded with P. fermentans WC 1507 cells. The beads were utilized immediately after preparation (panel A) and after washing with 9 g/L NaCl (panel B). Two consecutive runs are reported, the fresh medium being added in correspondence with the arrows. Xylose, blue; xylitol, red. The values are means of independent triplicates, SD always < 10%.*

### 3.2 Bioreactor experiments

Since washing the beads successfully preserved their xylitol-producing activity, the saline-washed beads were tested in stirred tank bioreactor. The fermentation was carried out under the conditions that were previously identified as optimal for xylitol generation (Ranieri et al. 2024a), specifically microaerophilic conditions obtained through suboptimal constant stirring and aeration, along with an acidic pH of 3.5. The stirring was kept constant at 800 rpm without cascade control to respond to oxygen consumption. Consequently, the DOT decreased below 5% in the first 12 h of fermentation and remained limiting throughout the process as required. At the same time, such stirring rate was adequate to preserve beads integrity, at least during the initial utilization cycles.
Consistent with flask-based observations, the first run was the least efficient, leading to the consumption of 86 g/L xylose and to the generation of 48.9 g/L xylitol in 288 h (Fig 3A). In the second and the third runs the consumption of xylose was 85 and 91 g/L, and the xylitol produced was 54.2 and 55.2 g/L, after 432 and 622 h, respectively. Throughout the process, $Y_{P/S}$ reached its maximum value of 65.2% during the second run, while in the third and first runs it settled to lower values, 60.8 and 57.1%, respectively (Fig 3A). The volumetric productivity improved from the first to the second run, increasing from 169.8 to 374.1 mg/L/h, before slightly declining to 326.0 mg/L/h during the third run.

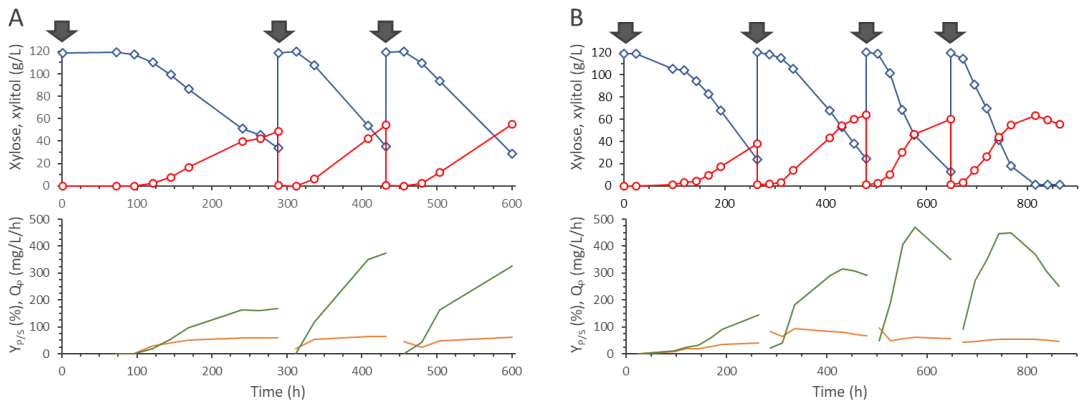


*Figure 3: Time-course of bioreactor fermentation runs with PVA beads washed with saline solution (A) or glycerol (B) before being introduced in the vessel. Repeated consecutive runs are reported, the fresh medium being added in correspondence with the arrow. Xylose, blue; xylitol, red; $Y_{P/S}$, orange; $Q_P$, green. The values are means of independent triplicates, SD always < 10%.*

At the end of the third run, the saline-washed beads resulted mostly damaged or busted, discouraging further use in fermentation runs. To address this, a 150 g/L glycerol solution was tested as an alternative washing medium. It was hypothesized that the hypertonic glycerol solution would mitigate water influx into the beads caused by osmotic effects during saline washing. Additionally, glycerol could act as a cryoprotectant, enabling bead freezing for storage or the application of freeze-thaw cycles to enhance PVA crystallinity (Sun et al., 2020). As a result, the beads treated with the glycerol solution were opaque and hard, with a diameter of 4–5 mm (Fig 1d). These beads were evaluated over 4 consecutive runs in stirred tank bioreactor (Fig 3B). Notably, the PVA beads maintained their shape and integrity without significant swelling throughout the 4 runs, suggesting their potential for further extended use.

Consistently with previous experiments, the 4 consecutive runs proceeded with increasing efficiency in terms of production titre and duration. In the first run, 94 g/L of xylose were consumed, yielding 37.9 g/L of xylitol in 264 h. In the second, the same amount of xylose was consumed, yielding 63.8 g/L xylitol in 216 h. In the third run 107 g/L were consumed in 168 h and 60.1 g/L of xylitol were produced, while in the fourth xylose was depleted in192 h to yield 59.6 g/L xylitol.

Correspondingly, volumetric productivity ($Q_P$) increased across runs (Fig 3B). $Q_P$ was the lowest in the first run (143 mg/L/h), then it increased substantially in the second run (317 mg/L/h) and reached the highest values in the third and the fourth runs (470 and 456 mg/L/h respectively). The conversion yield $Y_{P/S}$ also improved, starting at 40% in the first run and settled to 66, 55 and 53% in the following ones. These performance metrics were comparable to those obtained in airlift bioreactor using the same yeast immobilized in alginate beads (Ranieri et al., 2024a). However, PVA beads demonstrated to be a robust biomass carrier, that confirmed to be mechanically superior to alginate beads (Takei et al. 2011) and proved to be suitable for use in stirred systems. Stirred tank bioreactors provide superior mixing, enhanced mass transfer, and greater operational flexibility compared to pneumatic bioreactors (Fontana et al., 2009), while also offering a broader range of options for process optimization.

Interestingly, a decrease in xylitol accumulation rate was observed toward the end of each fermentation run, with a net decrease in xylitol titer occurring after xylose depletion. This behaviour suggests the occurrence of a diauxic shift, where xylitol generation competes with its respiratory utilization. This competition likely depends on the NADP+/NAPDH ratio and the oxygenation rate (Moysés et al., 2016; Zha et al., 2021). Therefore, a through optimization of the oxygen transfer rate (i.e., acting on the stirring and the aeration flow) could promisingly improve both the production rate and the conversion yield.

Future optimization efforts should also focus on the immobilized cell density within the beads, aiming to maximize viability and biocatalytic efficiency. A thorough assessment of yeast viability during both immobilization and fermentation phases will be critical for achieving these goals.

## 4. Conclusions

If prepared with the adequate precautions to preserve yeast viability, PVA beads proved to be a robust biomass carrier suitable for use in stirred-tank bioreactors. In this study, despite non-optimized process conditions, efficient xylitol production from xylose was achieved using PVA beads loaded with cells of *P. fermentans* WC1507, reaffirming its status as an excellent xylitol-producer. The immobilized cells were successfully utilized over four consecutive production cycles, without significant deterioration, highlighting the potential for further optimization of both s immobilization and process conditions. These promising results could lead to future perspectives in the biotechnological valorization of the pentoses obtained from plant biomasses as renewable feedstocks.

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