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Production of D-galactose using β-galactosidase and *Saccharomyces cerevisiae* entrapped in poly(vinylalcohol) hydrogel

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ABSTRACT

D-Galactose was produced from lactose (200 g l^{-1}) in the batch mode of a simultaneous saccharification and fermentation process (SSF). β-Galactosidases (from *Kluyveromyces lactis* and *Aspergillus oryzae*) and yeasts were immobilized in poly(vinylalcohol) hydrogel lens-shaped capsules – LentiKats®. After 20 repeated batch runs with entrapped *K. lactis*, β-galactosidase and free *Saccharomyces cerevisiae* (10% v/v inoculum), galactose productivity decreased to 50% and 1.4 kg of galactose were prepared. Compared to this, just 20% decrease of galactose productivity and a 0.9 kg production of galactose were observed for the SSF process with β-galactosidase from *A. oryzae* after 15 repeated batches under the same conditions. In the process of SSF with co-immobilized enzyme from *K. lactis* and *S. cerevisiae*, the galactose productivity increased from 3 g l^{-1} h^{-1} to 4.1 g l^{-1} h^{-1}, which reduced the time of preparation of D-galactose.

1. Introduction

D-Galactose, a monosaccharide found mostly in milk, has many applications, e.g. as pharmaceutical intermediate for several medicines, or as a stabilizer in intravenous solution for medical use. In the food industry, is widely used as a raw material for the manufacture of many sweeteners, such as arabinose, pentitol, arabinitol, globotriose, arbitol, galactitol, xylitol and tagatose (Granstrom, Takata, Tokuda, & Izumori, 2004; Ibrahim & Spradlin, 2000). Galactose is also useful in cell culture media as a nutrient or as an inducer in fermentations. Moreover, it can be used in the beverage industry, e.g. in sport drinks, in the manufacturing of contrast agents or as a substitute for phenols in resins (Darge, 2008; Ohlerth & O'Brien, 2007; Stannard, Hawke, & Schnell, 2007).

D-Galactose can be prepared from vegetable raw materials containing homologous (e.g. galactan) or non-homologous galactose polymers (e.g. legume material) (Molle & Kempener, 2000). The most common source of D-galactose is lactose (milk sugar), which is available cheaply and in large quantities as a by-product of the dairy industry. The process involves hydrolysis of lactose by enzymatic (Clyne & Wright, 1999) or chemical hydrolysis (Rosenberg, 2000), separation and purification of galactose. Galactose can be separated from a mixture of galactose and glucose by selective fermentation of the glucose to ethanol, using various kind of microorganisms (Cipolletti et al., 2007), or by glucose oxidase transformation of glucose into gluconic acid (Clyne & Wright, 1999).

To reduce the cost of D-galactose preparation, yeasts and enzymes were immobilized to poly(vinylalcohol) (PVA) gel particles LentiKats® in this study. Due to their shape (lenticular, diameter 3–4 mm, thickness 200–400 μm), LentiKats® have several advantages, e.g. superior mass transfer properties and easy separation from broth by sieves. In addition, they are prepared by a gentle immobilization method in an elastic PVA matrix known for its low toxicity, mechanical and good long-term stability and low biodegradability (Ding & Vorlop, 1995; Parascandola, Branduardi, & De Alteriis, 2006; Rebroš, Rosenberg, Mlichová, & Krištofíková, 2007). LentiKats® have been succesfully used for entrapment of cells, e.g. *Zymomonas mobilis* (Rebroš, Rosenberg, Stloukal, & Krištofíková, 2005), yeasts (Bezbradica, Obradovic, Leskosek-Cukalovic, Bugarski, & Nedovic, 2007) and enzymes, e.g. β-galactosidase (Grosová, Rosenberg, Rebroš, Šipozc, & Sedláčková, 2008) and glucoamylase (Rebroš, Rosenberg, Mlichová, Krištofíková, & Palúch, 2006).

The aim of our work was to examine the potential application of the non-aggressive LentiKats® technique for enzyme and cells immobilization in poly(vinylalcohol) in the simultaneous process of D-galactose production.

2. Materials and methods

2.1. Materials

Saccharomyces oviformis RIVE V 10-25-23 was obtained from the CCY collection of the Slovak Academy of Sciences in Bratislava. Microorganisms were stored at 4 °C, pH 6.8, on the medium,

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consisting of 20 g l^{-1} of glucose (Mikrochem, Slovakia), 10 g l^{-1} of yeast extract (Biospringer, France), 5 g l^{-1} of mycological peptone (Oxoid, England) and solidified with agar (20 g l^{-1}, Oxoid, England).

Saccharomyces cerevisiae was obtained from the industrial distillery, Kolín, Czech Republic. Microorganisms were stored at 4 °C, pH 6.8, on the propagation medium (described in Section 2.2) solidified with agar (20 g l^{-1}, Oxoid, England).

Aspergillus oryzae β-galactosidase G5160 was obtained from Sigma (USA, lyophilised powder, 8 U mg^{-1}).

Kluyveromyces lactis β-galactosidase 3000 L HP-G (Lactozym®) was obtained from Novozymes (Germany, liquid form, 85 U mg^{-1}). In this work, one unit (U) was defined as the amount of enzyme able to convert 1 g of lactose per hour to form glucose and galactose at 30 °C and pH 4.5 (*A. oryzae* enzyme) or pH 6.5 (*K. lactis* enzyme). The specific enzyme activity was defined as the enzyme activity per 1 ml of free enzyme (U ml^{-1}) or per 1 g of immobilized enzyme (U g^{-1}). The relative activity was calculated as the ratio of actual to maximum activity.

Poly(vinylalcohol) (PVA 17-99), and polyethylene glycol were provided by LentiKats Inc. (Praha, Czech Republic). Food lactose was delivered by Milei (Germany). Other chemicals were of analytical grade and were delivered by Lachema Ltd. (Brno, Czech Republic) or Mikrochem Ltd. (Pezinok, Slovakia).

2.2. Media

2.2.1. Production medium

The medium consisted of 200 g l^{-1} of lactose (Milei, Germany), 1 g l^{-1} of yeast extract (Biospringer, France), 1 g l^{-1} of $(NH_4)_2SO_4$, 1 g l^{-1} of KH_2PO_4, 0.5 g l^{-1} of $MgSO_4 \cdot 7H_2O$. The pH was adjusted to 4.5 or 6.5 (by 2 M KOH).

2.2.2. Inoculation and propagation medium

The medium consisted of 100 g l^{-1} of glucose (Mikrochem, Slovakia), 5 g l^{-1} of yeast extract (Biospringer, France), 5 g l^{-1} of $(NH_4)_2SO_4$, 1 g l^{-1} of KH_2PO_4, 0.5 g l^{-1} of $MgSO_4 \cdot 7H_2O$. The pH was adjusted to 6.5.

All media were autoclaved.

2.3. Preparation of biomass for immobilization

Inoculum was prepared in two steps. At first, 50 ml of inoculation medium (100 ml Erlenmeyer flask) were inoculated with *S. cerevisiae* and cultivated statically (24 h) at 30 °C. The biomass was then used as inoculum (5% v/v) in 250 ml of inoculation medium (500 ml Erlenmeyer flask) and cultivated under the same conditions.

Prepared inoculum (5% v/v) was grown in 1.5 l of inoculation medium (3 l Erlenmeyer flasks) statically (24 h) at 30 °C. Cells were harvested in the exponential phase (OD_{600} 2) by centrifugation (1200*g*, 15 min) and suspended in a solution of NaCl (8.5 g l^{-1}).

2.4. Immoblization of enzymes and cells

The immobilizations of *S. cerevisiae* cells and β-galactosidases were performed on the pilot-scale by LentiKat's Inc. (www.lentikats.eu, see also Ding and Vorlop (1995)). Biomass (0.7 g of the cells suspended in 50 ml of solution of NaCl (8.5 g l^{-1})) or 50 ml of enzyme (*A. oryzae* β-galactosidase (0.3 g ml^{-1}) was dissolved in 0.1 M acetate buffer, pH 4.5, mixed with 1 l of PVA gel (100 g l^{-1}). The mixture was extruded through thin nozzles onto a hard surface and dried in a laminar airflow cabinet to 30% of the initial mass. Solid gel particles (LentiKats®) were swollen in stabilizing solution for 45 min according to the manufacturer's instructions.

2.5. Propagation of cells in LentiKats®

Biomass (*S. cerevisiae*) immobilized in LentiKats® (described above) was propagated in a stirred-tank bioreactor filled with 3 l of propagation medium and approximately 400 g of immobilizates at 30 °C with agitation (200 rpm). When the glucose utilization reached 90%, medium was separated and LentiKats® were washed with distilled water and transferred into fresh propagation medium. After eight cycles, the immobilizates, with biomass concentration of 160 mg in 1 g of LentiKats®, were used in all experiments.

2.6. Batch fermentations on hydrolyzed lactose

Erlenmeyer flasks (500 ml) containing of 270 ml of production medium with hydrolyzed lactose (200 g l^{-1}, prepared externally, reaching almost 80% of conversion by immobilized *K. lactis* β-galactosidase) were inoculated with 10% (v/v) of inoculum (*S. cerevisiae* or *S. oviformis*, both prepared as described in preparation of biomass for immobilization part). Fermentations were carried out at 30 °C and pH 6.5 with addition of 0.4 g of $CaCO_3$ at the beginning of the processes. The reaction mixture was agitated (130 rpm) with a magnetic stirrer. Experiments were duplicated.

2.7. SSF with free cells and enzyme

A fermentor (1.3 l) containing 0.8 l of production medium (lactose 200 g l^{-1}) was inoculated with *S. cerevisiae* inoculum (10% v/v, prepared as described in preparation of biomass for immobilization part) and *K. lactis* β-galactosidase (0.2%). SSF was carried out at 30 °C, pH 6.5 (automatic addition of 2 M KOH) and agitation of 200 rpm. Experiments were duplicated.

2.8. Repeated batch SSF

A fermentor (1.3 l) containing 0.8 l of production medium (lactose 200 g l^{-1}) was inoculated with enzyme and *S. cerevisiae* in free or immobilizate forms (specified in the graph legends). Fermentations were carried out at pH 4.5 (experiments with *A. oryzae* β-galactosidase) or 6.5 (experiments with *K. lactis* β-galactosidase, maintained by automatic addition of 2 M KOH), temperature of 30 °C and gently stirring (200 rpm). When lactose hydrolysis reached 90–100%, and almost all glucose was utilized, fermentation was stopped. After each batch cycle, 90% of the medium was separated and the rest (10%) served as inoculum for the next batch run (for the experiments with immobilized yeast, whole medium was separated).

2.9. Analytical assays

Free cells biomass in the medium was calculated from the correlation curve between the O.D. at 600 nm and dry cells weight. Final galactose amount was calculated from final galactose concentration in the reaction broth multiplied by the reaction volume. Concentrations of lactose, glucose, galactose and ethanol were determined by HPLC with a refractive index detector K-2301 (Knauer, Germany), Ionex column (Watrex 250 × 8 mm), Polymer IEX 8 μm H form (Watrex, Czech Republic) with 9 mM H_2SO_4 as mobile phase, at 50 °C and at flow rate 0.7 ml min^{-1}.

3. Results and discussions

3.1. Simultaneous saccharification and fermentation (SSF)

The process for galactose production from lactose consists of three main steps: hydrolysis of lactose, separation of D-galactose