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Crosslinked, cryostructured *Lactobacillus reuteri* monoliths for production of 3-hydroxypropionaldehyde, 3-hydroxypropionic acid and 1,3-propanediol from glycerol

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ABSTRACT

Crosslinked, cryostructured monoliths prepared from *Lactobacillus reuteri* cells were evaluated as potential immobilized whole-cell biocatalyst for conversion of glycerol, to potentially important chemicals for the biobased industry, i.e. 3-hydroxypropionaldehyde (3HPA), 3-hydroxypropionic acid (3HP) and 1,3-propanediol (1,3PDO). Glutaraldehyde, oxidized dextran and activated polyethyleneimine/modified polyvinyl alcohol (PEI/PVA) were evaluated as crosslinkers; the latter gave highly stable preparations with maintained viability and biocatalytic activity. Scanning electron microscopy of the PEI/PVA monoliths showed high density of crosslinked cells with wide channels allowing liquid flow through. Flux analysis of the propanediol-utilization pathway, incorporating glycerol/diol dehydratase, propionaldehyde dehydrogenase, 1,3PDO oxidoreductase, phosphotransacylase, and propionate kinase, for conversion of glycerol to the three chemicals showed that the maximum specific reaction rates were −562.6, 281.4, 62.4 and 50.5 mg/g_{CDW} h for glycerol consumption, and 3HPA (extracellular), 3HP and 1,3PDO production, respectively. Under optimal conditions using monolith operated as continuous plug flow reactor, 19.7 g/L 3HPA was produced as complex with carbohydrazide at a rate of 9.1 g/Lh and a yield of 77 mol%. Using fed-batch operation, 1,3PDO and 3HP were co-produced in equimolar amounts with a yield of 91 mol%. The monoliths embedded in plastic carriers showed high mechanical stability under different modes in a miniaturized plug flow reactor.

1. Introduction

Chemicals with more than one functional group including polyols, hydroxycarboxylic acids and hydroxyaldehydes have been identified as important targets for the chemical industry based on renewable resources. They are potential building blocks for industrial polymers as well as excellent versatile platforms for other chemicals. Examples include 1,3-propanediol (1,3PDO), 3-hydroxypropionic acid (3HP) and 3-hydroxypropionaldehyde (3HPA) that are formed as metabolic products of glycerol by different anaerobic microorganisms (Banner et al., 2011; Della Pina et al., 2011; Dishisha et al., 2014; Jong et al., 2012; Vollenweider and Lacroix, 2004; Werpy et al., 2004).

3HPA possesses antimicrobial activity against a wide range of microorganisms allowing it to be used as a preservative (Schaefer et al., 2010; Vollenweider and Lacroix, 2004). Additionally, it serves as a precursor for products like acrolein, acrylic acid, and acrylamide (Jong et al., 2012; Vollenweider and Lacroix, 2004) with varying applications such as in paints, coatings, alkyd resins, etc.

3HP is also considered to be potentially important for several chemicals and polymeric materials; the most important being acrylic acid used in the synthesis of water-compatible resins and super absorbent polymers, and poly-3-hydroxypropionate which is a thermostable biodegradable polyester (Banner et al., 2011; Burridge, 2010; Della Pina et al., 2011; Dishisha et al., 2015; Jong et al., 2012; Werpy et al., 2004). It has also antibacterial and nematocidal activity, low metal corrosivity, and lower toxicity than lactic acid, which facilitates its use in food, fertilizers and cosmetics (Banner et al., 2011; Schwarz et al., 2004; Sebastianes et al., 2012). 1,3PDO is a dihydric alcohol incorporated as a co-monomer in polyesters mainly with terephthalic acid for the manufacture of Sorona® fibers that are incorporated in carpets and apparel (Banner

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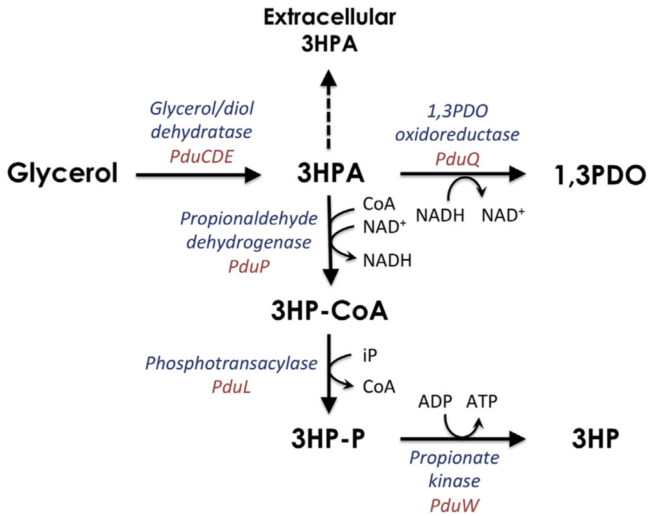

Fig. 1. Metabolic pathway for biotransformation of glycerol to 3HP and 1,3PDO via 3HPA as intermediate by resting cells of *Lactobacillus reuteri.* The different enzymes, intermediates and co-factors involved are shown.

et al., 2011; Werpy et al., 2004; Jong et al., 2012). Although 1,3PDO is industrially produced from fossil-based propylene (Haas et al., 1998; Powell et al., 1998) and more recently also from glucose by metabolically engineered bacteria (Laffend et al., 2000), no industrial process for production of 3HPA or 3HP has yet been established due to low economic feasibility and/or environmental benefits for most of the developed processes (Ohara et al., 2000; Vollenweider and Lacroix, 2004).

Lactobacillus reuteri is an organism known for its probiotic effect and recent reports have shown it to have potential as a biocatalyst for the emerging bio-based chemical industry (Vollenweider and Lacroix, 2004; Dishisha et al., 2015, 2014). This is largely attributed to its capacity for metabolism of glycerol using the 5 enzymes encoded in its propanediol-utilization (*pdu*) operon, namely glycerol/diol dehydratase, propionaldehyde dehydrogenase, phosphotransacylase, propionate kinase and 1,3PDO oxidoreductase (Sriramulu et al., 2008). Through these enzymes, dehydration of glycerol to 3HPA, and further conversion of 3HPA to 3HP and 1,3PDO was achieved using resting (non-growing) cells of *L. reuteri* (Fig. 1) (Dishisha et al., 2015, 2014; Krauter et al., 2012; Sardari et al., 2014, 2013a, 2013b; Yasuda et al., 2007). By determining the flux through the *pdu* pathway it was possible to manipulate the production process to yield mainly 3HPA or an equimolar mixture of 3HP and 1,3PDO (Dishisha et al., 2014). The presence of different functional groups on 3HP and 1,3PDO (hydroxyl and carboxylic acid) should allow their easy separation from the product mixture (Abraham et al., 2016; Burgé et al., 2016; Moussa et al., 2016). Alternatively, the resulting 1,3PDO can be quantitatively oxidized to 3HP through an additional step incorporating *Gluconobacter oxydans* (Dishisha et al., 2015; Zhao et al., 2015). Operating the entire biotransformation in aqueous medium using resting cells and achieving a quantitative conversion of glycerol (Dishisha et al., 2015) adds considerable strength to this process compared to others reported (Ashok et al., 2013; Borodina et al., 2015; Cie et al., 2012; Honjo et al., 2015; Kildegaard et al., 2016; Sankaranarayanan et al., 2014; Su et al., 2015) (Supporting Table S1 & S2).

Since several enzymes and cofactors are involved in the transformation of glycerol by *L. reuteri* (Fig. 1), the use of whole cells as a biocatalyst provides a more economical and robust system over using isolated enzymes. It also provides higher enzyme stability and allows simple co-factor regeneration. However, separation and recycling of the biocatalyst remains one of the limiting steps especially when biocatalyst reuse is crucial for process economy. Immobilization of the biocatalyst can help to overcome this problem. For several decades, immobilization of cells and enzymes has received considerable interest as a means to facilitate biocatalyst recycling and also improve its operational stability.

The common techniques for cell immobilization are adsorption to a solid support or entrapment within a polymeric gel (Klein and Ziehr, 1990; Nilsson et al., 1983). These techniques are characterized by dilution of the volumetric activity of the biocatalyst caused by the high volume occupied by the matrix in the bioreactor (both adsorption and entrapment), mass-transfer limitation and low mechanical stability (entrapment) (Karel et al., 1985), and low biocatalyst load which reduces the volumetric productivity (adsorption) (Klein and Ziehr, 1990). In general, these techniques have been successfully applied in the production of pharmaceuticals and specialty chemicals, however bulk chemicals with large market volume and low product price demand high volumetric productivity (Werpy et al., 2004).

Carrier-free biocatalysts, obtained by direct aggregation of cells or enzymes (CLEAs) using crosslinking agents, allow full use of all the available space within the bioreactor (Sheldon, 2010). Among the various crosslinking procedures, cryogelation involving crosslinking at sub-zero temperatures before thawing at room temperature, is superior in terms of providing structures with interesting physical and operational characteristics since they enclose wide channels facilitating mass transfer (Lozinsky et al., 2001, 2003). Recently, a novel technique for preparation of cryogels from whole cells using macromolecular crosslinkers has been reported (Aragão Börner et al., 2014; Zaushitsyna et al., 2014).

Here we present a model study on immobilization of *L. reuteri* cells as monoliths by crosslinking during cryostructuration and subsequent evaluation in a miniaturized plug flow bioreactor for transformation of glycerol, obtained as a by-product of biodiesel process (Perstorp AB), to 3HPA, 3HP and 1,3PDO.

2. Materials and methods

2.1. Materials

Dextran, technical grade T500 was a product from Pharmacia (Uppsala, Sweden), polyethyleneimine (MW 1800) was from Polysciences (Warrington, PA, USA), while glutaraldehyde (GTA) solution (50% v/v), potassium periodate (KIO_4), and poly(vinyl alcohol) (MW 89,000–98,000) were from Sigma-Aldrich (Steinheim, Germany). Bacto MRS broth was purchased from Difco Laboratories (Detroit, MI, USA). Standard 3HP (30% w/v) and glycerine Tech® (98%) were generous gifts from Perstorp AB, Sweden. All other chemicals were of analytical grade and obtained from commercial sources.

2.2. Microorganism and cultivation conditions

Lactobacillus reuteri RPRB3007 with a mutation in the catabolite repression element (CRE) upstream of the *pdu* operon (van Pijkeren et al., 2012) was used in the present study. Cultivation of the cells for use as biocatalyst was done as described elsewhere (Dishisha et al., 2014). Inoculum preparation was done in two stages in 30-mL serum bottles containing 20 mL medium composed of 55 g/L Lactobacilli MRS broth and 20 mM 1,2-propanediol. The first culture was incubated for 16 h at 37 °C and then used to inoculate another serum bottle, which was incubated for 8 h before inoculating a 3-L Applikon bioreactor (Microbial Biobundle, The Netherlands) containing 2 L of culture medium (55 g/L MRS, 5 g/L 1,2-propanediol and glucose concentration adjusted to 40 g/L) that was maintained under anaerobic conditions at 37 °C, 200 rpm stirrer speed, and pH