Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

2,3-Butanediol production using *Klebsiella oxytoca* ATCC 8724: Evaluation of biomass derived sugars and fed-batch fermentation process

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ARTICLE INFO

Keywords:
2,3-Butanediol
Lignocellulosic biomass
Hydrolyzate
Fed-batch fermentation

ABSTRACT

For this study, 2,3-butanediol (BD) fermentation from pure and biomass-derived sugar were optimized in shake-flask and 5-L bioreactor levels using *Klebsiella oxytoca* ATCC 8724. The results showed that 70 g/L of single sugar (glucose or xylose) and 90 g/L of mixed-sugar (glucose:xylose = 2:1) were optimum concentrations for efficient 2,3-BD fermentation. At optimum sugar concentrations, 2,3-BD productivities were 1.03, 0.64 and 0.50 $gL^{-1}h^{-1}$, and yields were 0.43, 0.36 and 0.35 g/g in glucose, xylose and mixed-sugar medium, respectively. The lack of simultaneous utilization of glucose and xylose led to the lowest productivity in the mixed-sugar medium. Detoxification of biomass hydrolyzates was necessary for efficient 2,3-BD fermentation when sugar concentrations in the medium was 90 g/L or higher, but not with sugar concentrations of 30 g/L or less. A fed-batch fermentation using glucose medium led to an increase 2,3-BD titer to 79.4 g/L and yields 0.47 g/g, while productivity decreased to 0.79 $gL^{-1}h^{-1}$. However, the fed-batch process was inefficient using mixed-sugar and biomass hydrolyzates because of poor xylose utilization. These results indicated that appropriate biomass processing technologies must be developed to generate separate glucose and xylose streams to produce high 2,3-BD titer from biomass-derived sugar using a fed-batch process.

1. Introduction

Biofuel and biochemical production using lignocellulosic feedstocks have gained increased attention because of their abundant availability, no direct competition with food supply, and environmental benefits [1,2]. Selection of appropriate lignocellulosic biomass feedstocks is critical for sustainable biofuel and biochemical production [3]. High-yield sorghum, switchgrass, and poplar are some of the major energy crops for the US because of their relatively high and reliable productivities across a wide geographical range, suitability for marginal quality land, and other positive environmental attributes [4]. Development of appropriate lignocellulosic biomass pretreatments to release clean sugar and efficient fermentation of these biomass-derived sugars to high-value fuels and chemicals are critical for sustainable bioconversion [3,5]. Production of bulk and specialty chemicals that are more expensive to produce by chemical process should be the focus of lignocellulosic-based biorefinery industries for economic viability. 2,3-Butanediol (BD) is one of the important platform chemicals with wide applications in fuels, chemicals, polymers, foods, and pharmaceuticals [6,7], and its synthesis by chemical methods is costlier than biological method [8]. Therefore, commercial production of 2,3-BD is currently viable only through biological routes. Global demand for 2,3-BD was estimated around 32 million tons (approximately $43 billion in sales) in 2011 [9]. 2,3-BD is a four-carbon alcohol that exists in three stereoisomers: L-(+)-2,3-BD (S,S − dextrorotatory form), D-(−)-2,3-BD (R,R − Levorotatory form), and *meso*-2,3-BD (optically inactive form). Microbial production of 2,3-BD was started more than 100 years ago, with intensified research during World War II because of the huge demand of 1,3-butandiene, a compound used at that time for synthetic rubber production and an important derivative of 2,3-BD [10].

2,3-BD can be produced by several microbial species such as *Bacillus, Klebsiella, Serratia and Enterobacter* genera using a wide range of feedstocks. Among these microbes, *K. oxytoca, B. licheniformis, K. pneumoniae, S. marcescents,* and *E. aerogenes* are the most promising microorganisms for efficient 2,3-BD fermentation [10]. Most of these microbes use mixed acid fermentation processes to produce 2,3-BD along with several byproducts as shown in Fig. 1 [7,11]. The end-products in each fermentation process vary based on the fermentation conditions and type of microorganisms. For example, most of the 2,3-BD producing microorganisms obtain energy from both fermentation and respiration pathways because they are facultative anaerobes. If the fermentation process is highly aerobic with a saturated oxygen level in

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http://dx.doi.org/10.1016/j.procbio.2017.05.001
Received 3 January 2017; Received in revised form 27 March 2017; Accepted 1 May 2017

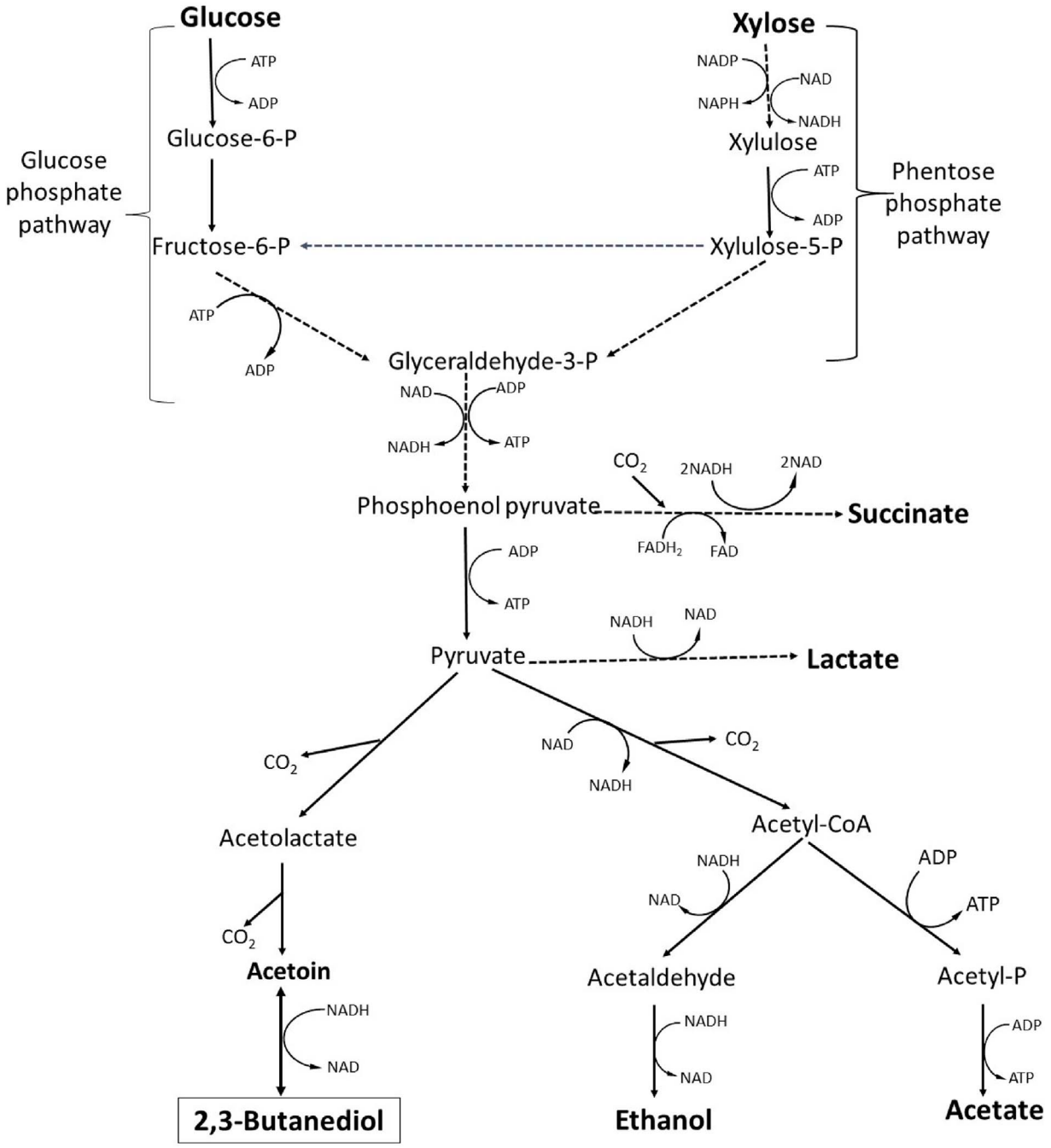

Fig 1. Different metabolic routes of glucose and xylose metabolism during 2,3-buanediol fermentation [7,11].

the fermentation medium, the acetate production pathway is predominant, while lactate biosynthesis is predominant if an anaerobic condition is maintained. Intermediate levels of oxygen in the fermentation medium favor the production of 2,3-BD, ethanol and acetoin [7,12]. In addition, many other parameters such as substrate types and concentrations, temperature and pH also significantly affect the end-product formation [7]. Even under optimum operating parameters, the type of microorganism used in the fermentation will determine the different stereoisomers of 2,3-BD. For example, a racemic mixture of L-(+)-2,3-BD and *meso*-2,3-BD is produced by *K. oxytoca*, and a mixture of D-(−)-2,3-BD and *meso*-2,3-BD is produced by *B. subtilis*; while optically pure *meso*-2,3-BD is produced by *S. marcescens* [10].

The capability of exploiting 2,3-BD producing organisms to utilize biomass-derived sugars is critical for the commercial production of 2,3-BD. Glucose and xylose are the major fermentable sugars derived from lignocellulosic biomass at approximately a 2:1 ratio, respectively [13]. Among the various 2,3-BD producing microorganisms, *K. oxytoca* is a highly efficient bacterium, capable of using a wide range of feedstocks including glucose and xylose [14]. In addition, separation of 2,3-BD from fermentation broth is one of the major bottlenecks for commercially viable process development, because 2,3-BD separation involves 50% of the downstream processing cost [15,16]. A high 2,3-BD titer with low concentration of byproducts is desirable in a fermentation broth in order to reduce downstream processing costs. A high 2,3-BD concentration in fermentation broth is much less toxic compared with other products such as ethanol; this indicates a possibility of producing high 2,3-BD titer using appropriate fermentation processes [8,17]. Batch fermentation, however, is limited because of substrate inhibition at the high initial sugar concentrations required to achieve high 2,3-BD titer. Therefore, fed-batch fermentation is a better choice to achieve this