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Genetic engineering of *Clostridium acetobutylicum* to enhance isopropanol-butanol-ethanol production with an integrated DNA-technology approach

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ABSTRACT

Acetone being a non-fuel solvent produced during the traditional acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum*; reduces the overall fuel alcohol yield. However, the conversion of acetone into isopropanol has been recommended to improve the process economy. The present study aims to develop an engineered *C. acetobutylicum* DSM 792 strain to convert acetone into isopropanol by introducing the secondary alcohol dehydrogenase gene from *Clostridium beijerinckii* NRRL B593 using the allele-coupled exchange approach. Batch and continuous fermentation experiments were carried out with a modified strain *C. acetobutylicum* DSM 792-ADH to improve the isopropanol yield and titer. The growth and production behavior of the modified strain in stationary flask culture and controlled batch culture was studied. Almost 50% of acetone was converted into isopropanol with highest total solvent yield to be 0.39 g g^{-1} glucose. The modified strain also utilized sugar mixture and SO_2–ethanol–water spent liquor as a substrate to produce the solvents.

1. Introduction

The demand for a clean and renewable biofuel has dramatically increased as a new benchmark on the petroleum industry from last few decades. Butanol being a superior biofuel to ethanol has attracted an increased attention because of its chemical properties including higher energy density and less hygroscopicity [1]. However, the productivity and yields during biofuel production are limited by chaotropicity, which is reported to be extreme for butanol [2–5]. Butanol has traditionally been produced by acetone-butanol-ethanol (ABE) fermentation using clostridia [6]. However, ABE fermentation is currently considered as less economical process for biofuel production with respect to carbon recovery. The acetone produced during this process cannot be used as a fuel due to several reasons including its corrosiveness [7–11]. The co-production of acetone with butanol (and ethanol) is considered undesirable because of the reduction in butanol yield per unit mass of substrate utilized during the process [9]. Thus, a reduction in the acetone production has been an important objective of clostridial metabolic engineering. Hence, it is desirable to suppress the formation of acetone or to convert it into another fuel additive such as isopropanol. Isopropanol is a simple secondary alcohol that has been used as a cleaning agent and plasticizer in the plastics industry. Recently, it has been reported that isopropanol can also be used as a fuel additive for the preparation of high-octane gasoline [12,13].

Many bacterial metabolites released from bacterial cells, including alcohols, act as antimicrobials and they frequently presents an obstacle to biotechnologists [2,4,14]. Furthermore, the microorganisms used to carry out fermentations are already stressed by other factors including high sugar concentrations and temperature fluctuations [4,15,16]. However, isopropanol is considerably more polar and less chaotropic than butanol and, hence is typically less inhibitory to the bacterial cell [4,5,17]. The attempts to reduce the acetone production by metabolic engineering have so far resulted in decreased butanol production and large accumulation of acetic and butyric acids in the broth [7,10,11,18]. Acetone formation is essential in the cell culture for cytosolic detoxification from carboxylic acids and protons to increase the culture pH in response to acetic acid and butyric acid to produce butanol and ethanol [19]. Hence the conversion of acetone into isopropanol is desirable instead of suppressing the acetone production in metabolic pathway. The conversion of acetone into

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