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Escherichia coli wild type and hydrogenase mutant cells growth and hydrogen production upon xylose and glycerol co-fermentation in media with different buffer capacities

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ABSTRACT

In this study, the new strategy for long term bio-hydrogen (H_2) production using different substrates and waste materials is presented. Growth characteristics and H_2 production were investigated upon consumption of 0.4% xylose and 1% glycerol alone (which were optimal) or their mixture by *Escherichia coli* BW25113 wild type parental strain (PS) and Δ*hyaB*, Δ*hybC*, Δ*hycE*, Δ*hyfG* mutants with genes deletions for key subunits of hydrogenase (Hyd)-1 to Hyd-4, respectively, in high and low buffer capacity peptone (HPM, LPM) mediums, pH 5.5 and 7.5. Overall, pH 5.5 negatively affected bacterial growth and H_2 production. At pH 7.5, apart from Hyd-3 and Hyd-4 mutants, upon growth of PS, Hyd-1 and Hyd-2 mutants drop of Pt redox electrode readings from positive (~+150 mV) to negative (of −400 to −550 mV) values was detected during log growth phase mentioning H_2 formation. Xylose and glycerol co-utilization did not affect PS and Hyd-1 and Hyd-2 mutant's biomass and H_2 formation during log growth phase in LPM, but ~1.5 fold stimulated these parameters, especially in HPM, pH 7.5, during prolonged 96 h bacterial growth. Roles of Hyd-3 and Hyd-4 in H_2 production; and Hyd-1 and Hyd-2 in H_2 oxidation during bacterial log growth phase were stated under xylose and glycerol co-fermenting conditions. The results obtained might be valuable for industrial long term H_2 production by bacteria using mixture of carbon sources and combining various organic waste materials.

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Introduction

Production of clean energy sources such as bio-hydrogen (H_2) using of carbon rich containing wastes is new and perspective strategy to meet increasing energy demand [1]. Different types of organic wastes, including lignocellulose materials, can be used for H_2 production. The most important criteria for waste selection to produce H_2 are its accessibility, the presence of sugars (carbohydrates) or carbon rich other compounds,

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utilization of these sources by microbes and, at last, its value [2–5]. Unfortunately, organic wastes are not easily utilized by microbes and they cannot be directly applied for H_2 production. That is why it is required to develop and apply technologies for pre-treatment and processing of the waste [5–7].

It has been shown that the annual production of the lignin biomass on the Earth is over 200 billion tons and widely available [5]. Plant biomass and main component of plant cell walls is lignocellulose, which consists of 20–45% cellulose, 16–37% hemicellulose and 12–26% lignin [5–7]. These complex organic polymers can be utilized by microbes in very difficult manner and cannot be directly applied for bacterial biomass formation and H_2 production. It is required to pretreat the waste for lignin removal and hydrolysis of complex carbohydrates as well as to find out optimal dilutions for bacterial biomass formation and H_2 production [2,6]. It has been revealed that H_2 can be produced upon microbial growth on lignocellulosic biomass [2,7–10]. Xylose is among the most abundant major sugars derived from the breakdown of lignocellulosic biomass taking up to 30–40% of hydrolysates [6,9,11]. Taking into consideration that lignocellulosic biomass can be applied after some pretreatment and dilutions [6], it is likely to ignore the other minor components. Another attractive waste material for H_2 production is glycerol, the end material (by-product) of biodiesel and other industries [12–14]. Moreover, recently the cost of glycerol was intensely reduced [13,14].

It was stated that both glycerol and xylose as well as ethanol, acetate and other end products can be fermented by *Escherichia coli* and other microorganisms with the formation of H_2 [12–17]. However, pathways of glycerol and xylose fermentation by bacteria are relatively different: for example, glycerol exports into the *E. coli* cell mostly by passive transport trough GlpF glycerol facilitator protein and, under anaerobic conditions it is metabolized to the glycolytic intermediate dihydroxyacetone (DHA) phosphate (DHAP) (Fig. 1). Moreover, the conversion of DHAP to phosphoenolpyruvate and then to pyruvate is coupled to DHA phosphorylation, which generates a loop path in the metabolic pathway [16,17]. The exceed of reducing equivalents in the form of NADH are observed upon glycerol fermentation. As a consequence, generally ethanol as a main product of glycerol utilization was identified [16,17]. Glycerol can be fermented by *E. coli* at wide range of pH from pH 5.5 to pH 7.5 but most optimally at pH 6.3 [17,18].

Whereas D-xylose is transported into *E. coli* cells through different means, the main transport pathway is a high-affinity ABC transporter, XylFGH [19]. After entering into the cell, xylose is metabolized by isomerase pathway (see Fig. 1): xylose isomerase (XylA) and xylulokinase (XylB) convert xylose to xylulose-5-phosphate [20]. Then, xylulose-5-phosphate is converted by the enzymes of the pentose phosphate pathway (PPP) [21,22] before passing into the Embden-Meyerhof-Parnas pathway as fructose-6-phosphate and glyceraldehyde-3-phosphate [19] (see Fig. 1). Conversion of xylose to acetate and ethanol increases the net ATP yield from 0.67 to 1.5 per xylose [19,23]. Moreover, under energy limited conditions, it has been shown, pyruvate formate lyase (PFL) and acetate kinase (AK) are essential for anaerobic growth of *E. coli* on xylose [24].

In *E. coli* H_2 is produced from formate, the end product of both glucose and xylose or glycerol fermentation, due to operation of formate hydrogen lyase (FHL) (see Fig. 1). FHL composed of formate dehydrogenase H and membrane-bound [Ni–Fe]-hydrogenase (Hyd) enzymes [10,25,26]. Four Hyd enzymes (Hyd- 1–4) encoded by *hya*, *hyb*, *hyc* and *hyf* operons, respectively, are involved in H_2 metabolism [10,25–28]. These Hyd enzymes are reversely catalyzing H_2 formation and oxidation. These enzymes are studied intensively by different groups, their expression and activity have been well reviewed [10,25,26], however the findings are too complicated and sometimes controversial especially upon fermentation of different substrates and under different conditions. Therefore, new study of responsible Hyds under different conditions and their regulation is very urgent.

Actually, it has been shown that the activity and operation direction of Hyds depend on fermentation substrates and external conditions (pH, oxidation-reduction potential (ORP, Eh) etc.) [27,29–31]. Hyd-1 and Hyd-2 are mostly H_2-oxidizing enzymes, but their function is substrate dependent: during glucose fermentation both enzymes operate in H_2-oxidizing mode, while upon glycerol fermentation Hyd-2 can evolve H_2 [10,14,18]. Expression of the *hya* operon coding Hyd-1 is induced at acidic pH, whereas maximal expression of the *hyb* operon coding Hyd-2 was shown for alkaline pH in cultures grown on H_2 and fumarate. Moreover, Hyd-2 is more active under reducing conditions (Eh below −80 mV), while Hyd-1 is active under oxidizing conditions. Both these Hyds are suggested to be not required for anaerobic growth but they are required for the response to a shift of external pH from alkaline to acidic conditions [28]. Hyd-3 and Hyd-4 operate as H_2-evolving enzymes during glucose fermentation, but these enzymes can operate in reverse mode during glycerol fermentation [10,25,26]. Hyd-4 is probably active at low concentration of glucose [31] and at alkaline pH [27]. Together, these four Hyd enzymes are suggested to perform H_2 recycling, and a metabolic cross-talk between Hyd enzymes is proposed [10].

During fermentation, the ORP of *E. coli* cultures has been demonstrated to decrease to low, negative values (up to ~ −550 mV), as determined with a platinum (Pt) electrode which is sensitive to H_2 under anoxic conditions [32,33]. These values of ORP are correlated with Eh for formate:H_2 couple which is −420 mV [27]. Furthermore, the ORP of the bacterial culture is also influenced by the ratio of oxidized to reduced fermentation products, as well as on pH based of the Nernst equation: $Eh = E_0 + (RT/nF) \ln([ox]/[red]) + (RT/nF) \ln [H^+]$, whereby as the Eh decreases, the pH increases. However, the interrelationship between an observed decreasing in ORP with the lowering of pH during bacterial growth under anaerobic conditions is not simple, and it is difficult to explain; this is mostly because of the complexity of redox processes going on during fermentation, including redox processes within bacterial membrane and H_2 cycling via Hyd enzymes, which cannot be described by the equation beyond. ORP has been shown can determine bacterial growth under fermentative conditions: its reducing values stimulate fermentation [33,34], and this parameter will be influenced by the activities of Hyd enzymes, as well as other membrane-associated oxidoreductases that affect proton release and pH. This relationship between ORP and Hyd enzyme activity, though, is very important for bacterial growth under fermentation and H_2 production but it is poorly understood.

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