

and the product isolation is complicated. To overcome these limitations, enzymatic synthesis could be considered.  $\alpha$ -Ketoglutarate can be synthesized by deamination of cheap L-glutamate by glutamate dehydrogenase. For the enzymatic synthesis, NAD<sup>+</sup>-dependent glutamate dehydrogenase from recombinant *Escherichia coli* which contains thermophilic glutamate dehydrogenase from *Thermus* sp. was purified for the enzymatic synthesis of  $\alpha$ -ketoglutarate. The purified enzyme was characterized and studied kinetics. Then,  $\alpha$ -ketoglutarate was produced by using purified glutamate dehydrogenase combined with electrochemical reaction system to regenerate NAD<sup>+</sup>. Continuous type of electrochemical bioreactor was designed for the continuous  $\alpha$ -ketoglutarate production. For this setup, cofactor was modified with polyethylene glycol(PEG) to retain in the reactor for the continuous process. Details will be presented and discussed.

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## BR-P6

### Treatment of water hyacinth for bioethanol production

Bong Je Park, and Hyun Shik Yun

Department of Biological Engineering, Inha University, Incheon, Republic of Korea

The world demand of ethanol as an alternative fuel for petroleum is increasing rapidly because of high oil price, fossil fuel exhaustion and global climate change. Water hyacinths are used widely for the improvement of water quality by removing nutrients and heavy metals. However, they are also becoming a problem in lakes, ponds and waterways in many parts of the world. Consequently, water hyacinths are the next promising renewable energy resource. The ethanol production from biomass consists of pretreatment, enzyme hydrolysis, fermentation and product separation. Pretreatments are necessary to improve the digestibility of the lignocellulosic biomass. The main effects are dissolving hemicellulose and alteration of lignin structure by providing an improved accessibility of the cellulose for hydrolytic enzymes. The effectiveness of acids, alkalies, oxidatives, and thermal pretreatments for conversion of water hyacinth to ethanol was investigated. The conversion of cellulose and hemicellulose to monomeric sugars was done enzymatically by addition of cellulases and hemicellulase. The produced monomeric sugars was fermented to ethanol by *Saccharomyces cerevisiae*.

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## BR-P7

### Succinate production from glycerol with acetate as an electron sink

Shigetsugu Sakai, Hiroyuki Arai, Masaharu Ishii, and Yasuo Igarashi

University of Tokyo, Tokyo, Japan

Glycerol is an inexpensive and abundant unused resource produced as a byproduct in biodiesel industry. Because of its higher reducing state compared to common sugars such as glucose, glycerol is appropriate for succinate production.

In this study we focused on succinate production from glycerol in *Escherichia coli*. Redox-balanced fermentation products from glycerol are limited to ethanol or succinate in *E. coli* and it is known that wild-type *E. coli* mainly produces ethanol from glycerol.

We constructed pflB/ldhA double deletion mutant for elimination of undesired byproducts such as ethanol, acetate and lactate. This strain mainly produces succinate from glycerol under the presence of organic nitrogen sources. Although the mutant could not produce succinate under inorganic medium supplemented with glycerol, productivity was recovered by adding acetate to the medium under microaerobic condition. A main byproduct was ethanol in this condition and additional adhE gene deletion for elimination of ethanol resulted in complete lost of succinate productivity. This process required relatively high initial concentration (100 mM) of acetate in comparison with acetate consumed (about 30 mM) and we thought it might because of low affinity for acetate of Pta–Ack pathway which played a main role for acetate utilization under anoxic condition. Overexpression of acs gene coding a high affinity acetate utilization pathway resulted in promotion of succinate production under conditions with low initial concentration (10 or 30 mM) of acetate.

As a result of <sup>13</sup>C stable isotope experiment, it was shown that succinate was produced from glycerol plus CO<sub>2</sub> and that acetate was converted mainly to ethanol for re-oxidation of NADH. Detailed analysis of fermentation products revealed that pyruvate was produced as a byproduct and it was indicated that NADH re-oxidation via acetate–ethanol conversion is needed for the maintenance of redox state caused by pyruvate accumulation.

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## BR-P8

### Effect of moderate pressure on the production of hydrocortisone in an *Absidia coerulea* bioconversion system

Shiru Jia, Changsheng Qiao, Xu Xu, and Zhilei Tan

Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, China

In recent years, different features of microbial transformation of 17 $\alpha$ -hydroxypregn-4-en-3, 20-dione-21-acetate (RSA) to hydrocortisone (HC) have been investigated (1). However, the production of HC from RSA was limited because of the relatively low solubility of RSA and O<sub>2</sub> in water during the bioconversion. There is a growing interest in new methods to improve the solubility of RSA and O<sub>2</sub>. This study developed a new method for the improvement of the production of HC from RSA by moderate pressure in a bioconversion system. The effects of the moderate pressure (0.1–2.5 MPa) on viability, cell membrane permeability and catalyzing activity of *Absidia coerulea* for RSA were investigated. The results showed that the shape of *A. coerulea* mycelium became looser and cell membrane permeability of *A. coerulea* mycelium was improved in moderate pressure, meanwhile, the viability of *A. coerulea* mycelium was kept at a high level. The yield of HC was improved over 1.25-fold compared with that of the control (untreated cells), and came up to 350 mg/L when *A. coerulea* mycelium was treated under 0.5 MPa of pressure with atmosphere