High-Density Cultivation of Hyperthermophilic Archaea

Sun Bok Lee, Chan Beum Park[1], Kee Woung Kim[2], and Eun Sung Kan[3]

Department of Chemical Engineering, Pohang University of Science and Technology, Korea, [1]Department of Chemical and Materials Engineering, Arizona State University, USA, [2]LG Life Science R&D, Seoul, Korea, [3]Department of Chemical Engineering and Environmental Engineering, University of California, Riverside, USA

TEL: +82-54-279-2268, FAX: +82-54-279-2699

ABSTRACT

The great diversity of hyperthermophilic archaea discovered to date has generated interest in a variety of potential applications involving the production of thermostable enzymes and the synthesis of biochemical compounds unique to these microorganisms. However, difficulties in cultivation have limited physiological studiesand practical application of these hyperthermophiles. Thus, the development of strategies to obtain high cell density under high temperature condition are of particular importance in increasing biomass yield of these microorganisms in an environment different from their natural habitat. To challenge this, we have studied the factors that affect the growth of *Sulfolobus solfataricus*, a thermoacidophilic archaeon which normally grows at 80C and pH 3, and *Aeropyrum pernix*, a marine hyperthermophilic archaeonwhich grows under strictly aerobic conditions at temperatures up to 100C. When hyperthermophilic archaea are cultivated under aerobic conditions, the loss of the culture volume becomes significant due to vaporization of water with aeration. To eliminate the problems caused by evaporation of the medium, we have devised a constant-volume fed-batch system, where the loss of water due to evaporation is compensated by feeding additional water into the fermentor. When this method was applied to high-density cultivation of *S. solfataricus*, the maximum cell density obtained was 22.6 g/L, which is the highest value reported for hyperthermophiles in fed-batch operations. In the next, the physiological and environmental factors that limit the efficient growth of hyperthermophiles were investigated. During fed-batch cultivation of *S. solfataricus*, we found thatprolonged incubation of L-glutamate under culture condition resulted in the conversion of L-glutamate to L-pyroglutamate and that L-pyroglutamate acted as a potent inhibitor against the growth of *S.*

solfataricus. Chemical modification of culture medium at high temperature also occurred during cultivation of *A. pernix*. In the case of *A. pernix*, the maximum cell density obtained with a constant-volume fed-batch system was only 4.5 g/L. We observed the Maillard browning reaction between sugars and amino acids contained in the medium and a significant growth inhibition by Maillard reaction products accumulated in the culture medium. We also found that, unlike *S. solfataricus*, *A. pernix* could not grow without an exogenous supply of adenine and several amino acids.