CH₄ PRODUCTION FROM H₂ AND CO₂ BY METHANOBACTERIUM THERMOAUTOTROPHICUM CELLS FIXED ON HOLLOW FIBERS

Hae Sung Jee, Naomichi Nishio, and Shiro Nagai

Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Saijo-cho, Higashi-Hiroshima 724, Japan

SUMMARY: Methane was produced from H₂ and CO₂ by Methanobacterium thermoautotrophicum cells fixed on the surface of hollow fibers. The mineral solution permeated through the inside of fibers was consumed by the cells, while the gaseous substrate flowing outside the fibers was directly metabolized to methane. Methane production was proportional to hollow fiber length i.e., contact area between cell layer and gas phase. In repeated batch cultures, the production rates of methane and cell mass were 33.1 L/L reactor/day and 1.75 g cells/L reactor/day, respectively with 90% conversion rate.

INTRODUCTION: Within the last ten years, immobilization techniques for whole cells on or in polymerized materials have extensively progressed in continuous biochemical processes (Chibata et al., 1977; Nishio et al. 1985). With immobilization techniques, a high cell density can be obtained easily, but for fermentations using gaseous substrates of low water solubility, such as H₂ or O₂, the supply of gaseous substrate becomes a growth limiting factor. A bioreactor consisting of a bundle of hollow fibers may provide a better supply of gaseous substrate to the growing cells (Fouron, 1987). For methane production from H₂ and CO₂ by the methanogen M. thermoautotrophicum, efficient CH₄ production could be achieved by fixing the methanogen cells on a solid support such as porous ceramic (Jee et al. 1987; Jee et al. 1988); however, in long term operation the accumulation of methanogen cells on the support hindered the homogeneous flow of the gaseous substrates through the pores of the support and this caused a gradual decrease of methanation from H₂ and CO₂ (Jee et al. 1988). In this work, a hollow fiber bioreactor of M. thermoautotrophicum cells fixed on the surface of hollow fibers was constructed and an efficient biomethanation of H₂ and CO₂ was attempted maintaining a high cell density for a long-term operation.

MATERIALS AND METHODS
Microorganism: The methanogen used was Methanobacterium thermoautotrophicum ΔH (DSM 1053). The microorganism was cultured on a modified medium (Schönheit et al. 1980).
Bioreactor: A hollow fiber module (Fus-3081, Daicel Chem. Ind., Co., Tokyo, Japan) for separation use was used for a bioreactor. This consists of a bundle of hollow fibers (hydrophilic polyether sulfone) (see Fig. 1). These fibers were fixed at both ends with epoxy resin and set in a cylindrical polycarbonate column, (22 mm

243
I.D. X 273 mm length). The reactor has 100 fibers and the volume fraction of the fibers to the reactor is 35%. The specification of the hollow fiber is as follows: length, 273 mm; O.D., 1.3 mm; I.D., 0.8 mm; average pore size of the surface of the fiber, 0.3 - 0.5 μm. The medium was supplied through the inside of the hollow fibers by a peristaltic pump at a flow rate of 15 ml/h. The methanogen cells fixed on the surface of the hollow fiber can utilize the medium which permeated through the hydrophilic fiber, while the gaseous substrate (H2/CO2 = 80/20, v/v) prepared with two mass flow controllers was fed directly to the outside of the fiber (see Fig. 1). Hence, the methanogens fixed on the fiber surface might utilize the gaseous substrate like the usual surface cultures. In preliminary experiments, reactors consisting of 1 (13.4 cm), 3 (35.7 cm) and 7 fibers (65.1 cm) were constructed to investigate the feasibility of this principle.

Culture: Preculture and stock culture were done in a 125-ml vial (20 ml medium), and culture techniques and conditions were reported previously (Jee et al. 1987). To stick the methanogen cells on the surface of fresh hollow fibers, the pre-cultured broth of a vial was fed to the bioreactor by a syringe and filtered through the fibers at a pressure difference (1.0 kg/cm²) with the mixed gas between the inside and outside of the fibers. Then, the cultivation was started supplying the fresh medium and the gaseous substrate was fed separately (see Fig. 1). At the end of each run, the cells grown on the fiber surface were harvested by the medium permeation under a slightly positive pressure, if necessary. After releasing the cells, the residual cells (ca. 38 mg) in the reactor (67.5 ml working volume) were again used as a seed culture for repeated batch culture. The cultivation was conducted at 65°C under atmospheric pressure, and pH of the medium was adjusted at 7.0 by adding 1 M Na2CO3.

Analysis: Gas composition of H2, CO2 and CH4 of exhaust gas from the reactor was analysed by gas chromatography as described previously (Jee et al. 1987). CH4 production rate was calculated from the gas flow rate and CH4 composition of the exit gas. The cells harvested from the reactor were centrifuged, washed once with distilled water and measured after drying at 105°C for 24 h.

RESULTS AND DISCUSSION

Preliminary tests: In the methanation of H2 and CO2, CH4 production can be increased by increasing the volumetric mass transfer coefficient of the substrate gas. Therefore a fermentor having high CH4 productivity can be constructed by increasing interfacial area between liquid and gas (H2 + CO2). In preliminary tests for CH4 production from H2 and CO2 using the new bioreactor, three reactors having 1, 3 and 7 fibers were comparatively tested for CH4 production. On each run, 6 mg cells/m fiber was fixed on the outside surface of the fiber. The feed rates of