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Study on Methane Fermentation and Production of Vitamin B_{12} from Alcohol Waste Slurry

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Abstract

We studied biogas fermentation from alcohol waste fluid to evaluate the anaerobic digestion process and the production of vitamin B_{12} as a byproduct. Anaerobic digestion using acclimated methanogens was performed using the continuously stirred tank reactor (CSTR) and fixed-bed reactor packed with rock wool as carrier material at 55°C. We also studied the effects of metal ions added to the culture broth on methane and vitamin B_{12} formation. Vitamin B_{12} production was 2.92 mg/L in the broth of the fixed-bed reactor, twice that of the CSTR. The optimum concentrations of trace metal ions added to the culture liquid for methane and vitamin B_{12} production were 1.0 and 8 mL/L for the CSTR and fixed-bed reactor, respectively. Furthermore, an effective method for extracting and purifying vitamin B_{12} from digested fluid was developed.

Index Entries: Vitamin B_{12}; methane; acclimated methanogens; trace metal ions; rock wool.

Introduction

Vitamin B_{12} is an important cofactor in many biochemical reactions and is widely used in chemotherapy and animal feed. Until now, vitamin

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B_{12} was produced industrially only by microbiological means, using propionic bacterium cultivated with sugar. The conventional method results not only in low production of vitamin B_{12}, but also in remarkable organic acid inhibition *(1,2)*. Vitamin B_{12} production by methanogens may have the following advantages over conventional vitamin B_{12} production: (1) the concentration of vitamin B_{12} in the culture broth is 10 times greater than that using propionic acid–utilizing microbes *(3)*; (2) the main product, methane, does not inhibit growth of methanogens and could provide a high cell density culture system; and (3) methanol, CO_2, and acetic acid used as substrates are inexpensive, relatively stable, and renewable.

On the other hand, because of the low growth rate of methanogens compared to aerobic microbes, a long substrate residence time is required to make the fermentation facility larger. To maintain a high density of methanogens in the fermentation reactor, a method for immobilizing microbial cells onto various supports has often been used *(4,5)*. Mazumder et al. *(6)* attempted continuous fermentation of extracellular vitamin B_{12} compounds using a diatomaceous clay fixed-bed bioreactor. The concentration of vitamin B_{12} was about 4 mg/L, and total cell mass retained in the reactor was 39.6 g dry cell L^{-1} *(6)*. However, only a few research studies have examined the production of vitamin B_{12} from alcohol waste fluid using acclimated methanogens, and none of these studies were carried out from continuous methane fermentation with a fixed-bed bioreactor packed with rock wool. The aim of the present study was to investigate the optimum operating conditions including the effect of trace metal ions on the production of vitamin B_{12} compounds from alcohol waste fluid utilizing acclimated methanogens in a fixed-bed anaerobic digester.

Materials and Methods

Reactor System

A schematic of the experimental system is shown in Fig. 1. One liter of culture liquid was added to a 2.8-L cylindrical glass column reactor, and 300 mL of cylindrical carrier was packed with rock wool. All of the tubing connections, stoppers, and seals in the column were made of butyl rubber. To prevent photodecomposition of vitamin B_{12}, the outside of the whole device was covered with a vinyl sheet to shut out light. The digestion was carried out at 55°C and 20 d of hydraulic retention time (HRT).

Culture Medium and Reactor Operation

Microbial inoculum was collected from an urban waste treatment plant, and alcohol waste fluid was obtained from a local brew plant. Sugar content was used as an organic load index, and the feed of the alcohol waste fluid to the reactor was diluted to 5% sugar content (as glucose) with distilled water. The basal medium consisted of the following components (per liter of distilled water): 3.40 g of KH_2PO_4, 3.40 g of K_2HPO_4, 2.54 g of Na_2CO_3, 2.0 g of yeast extract, and 0.75 g of NH_4Cl. First, 400 mL of the alcohol waste

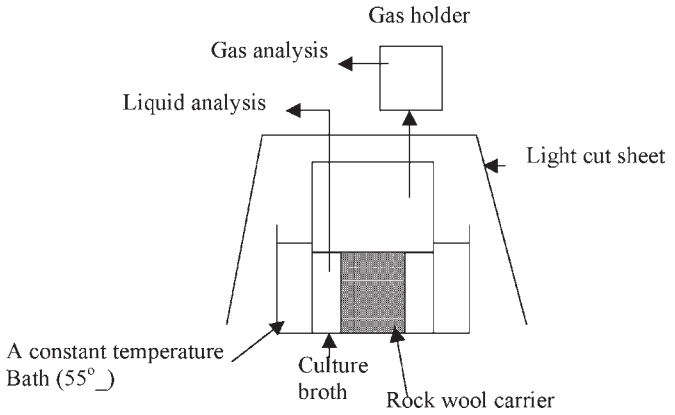

Fig. 1. Schematic of batch fermentation system with rock wool carrier.

Table 1
Composition of Trace Metal Solution

Compound	Concentration (mg/L)
$MgSO_4 \cdot 7H_2O$	600
$MnSO_4 \cdot H_2O$	5
$NiCl_2 \cdot 6H_2O$	125
$FeSO_4 \cdot H_2O$	28
$CoCl_2 \cdot H_2O$	10
$CaCl_2 \cdot H_2O$	40
$ZnSO_4 \cdot H_2O$	30
$CuSO \cdot H_2O$	8
$AlK(SO_4)$	1
H_3BO_3	1
$NaMoO_4 \cdot H_2O$	1.5

fluid and 400 mL of basal medium were added to the reactor, and then this was inoculated with 200 mL of inoculum to the reactor. To begin fermentation, the HRT of the alcohol waste fluid to the reactor at the initial stage of fermentation was set as 30 d, and then increased to 20 d gradually. The composition of trace metal solution is shown in Table 1.

Analysis Method

The concentrations of the gas-phase components were analyzed by a gas chromatograph (Shimadzu GC-8A; Kyoto, Japan) equipped with a thermal conductivity detector connected to a data analyzer (Shimadzu C-R4A Chromatopac). A stainless steel column packed with Porapak-Q was used.

The detector and injector temperature were both 60°C and the column temperature was 80°C. N_2 was used as the carrier gas at an inlet pressure of 199 kPa and outlet pressure of 150 kPa. This allowed very accurate determination of CO_2, H_2, and CH_4.

Vitamin B_{12} was analyzed by high-performance liquid chromatography (HPLC) (*[2]*; Jasco Culliver 1550) equipped with a Crest Pak C_{18} column at 40°C with an ultraviolet detector (355 nm). HPLC was performed with an automated gradient controller. KH_2PO_4 (10 m*M*) + $CH_3(CH_2)_5SO_3Na$ (2 m*M*) with an initial pH of 3.0 and methanol (100% [v/v]) were mixed as degassed solvent components A and B, respectively. The gradient condition was designed at response times of 0.0, 30.0, 35.0, and 35.1 min, and A/B ratios of 100/0, 50/50, 50/50, and 100/0. Solvents were used at a flow rate of 1 mL/min and at a maximum pressure of 46 kg/cm^2. The cyanocobalamin (Wako) was used as standard. A digital refractometer (ATAG; PR-101) was used to measure the sugar content as glucose.

Extraction and Purification of Vitamin B_{12}

Extraction and purification of vitamin B_{12} were performed as described by Sado *(8)*, Chanto et al *(7)*, and Mazumder et al. *(9)*. Finally, the following method modified by us was used throughout the study. A 4-mL sample of fermentation broth was centrifuged at 7000*g* for 4 min. The liquid (containing few or no bacterial cells) was directly passed through an Amberlite XAD-2 column (2 cm diameter × 10-cm length). After washing the column with water, the liquid with 0.01% (wt/v) KCN at pH 4.5 was passed through the same column once more and the absorbed corrinoids were changed to cyanocobalamin. The absorbed cyanocobalamin was eluted with 80% methanol containing 0.01% (wt/vol) KCN and 20% distilled water. The cyanocobalamin fraction was flash evaporated to dryness and dissolved in a small volume of water. In addition, vitamin B_{12} extraction and purification from a standard cyanocobalamin aqueous solution was tested using the modified method.

Results and Discussion

Extraction and purification of vitamin B_{12} were carried out by the method reported by Sado *(8)*; however, no vitamin B_{12} peak was determined by HPLC. Another method reported by Chanto et al. *(7)* showed that the culture broth was autoclaved for 10 min and then analyzed by HPLC. With this method, several peaks were present and the real peak of vitamin B_{12} could not be detected. It could not be isolated clearly because of determination errors. Then a modified isolation method of vitamin B_{12} was attempted using a column filled with Amberlite XAD-2 *(9)*. The details of the modified method was described above. A good recovery rate (about 90%) was obtained.

The effect of the concentration of trace metal ions on the conversion rate and vitamin B_{12} production was investigated first. As shown in the Figs. 2

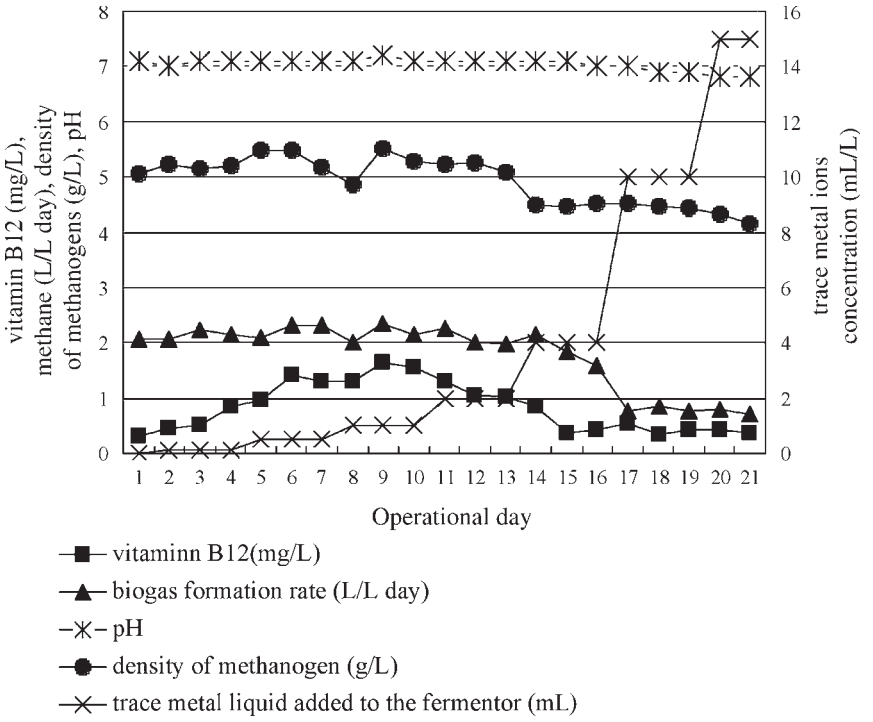

Fig. 2. Course of vitamin B_{12} production, methane production, and methanogen density with different trace metals ion added using a complete liquid stirred reactor.

and 3, the optimum concentration of trace metal ions added to the culture liquid for methane and vitamin production were 1.0 and 8 .0 mL/L for the CSTR and fixed-bed reactor, respectively. When fermentation was carried out at the optimum concentration of trace metal ions, the methane production rate and methanogen density were 2.35 L/(L·d) and 5.50 mg/L for the CSTR, and 3.90 L/(L·d) and 4.04 g/L for the fixed-bed reactor. Lower methanogen density in the broth of the fixed-bed reactor could be considered because most of the methanogens were fixed by rock wool. A corrinoid, such as vitamin B_{12}, containing cobalt ions is known to bind to coenzyme M (CoM) methylase, and therefore cobalt is essential for vitamin B_{12} synthesis *(10)*. To raise the production of vitamin B_{12}, the optimum trace metal ions added to the fermentation liquid should be a very important factor. As shown in the Figs. 2 and 3, inhibition of growth of methanogens occurred when the trace metal ion concentration was greater than 1.0 mL/L in the case of CSTR, and 8.0 mL/L in the fixed-bed reactor. At this concentration of trace metals ion, vitamin B_{12} yield and CH_4 formation decreased sharply. The maximum yield of vitamin B_{12} obtained was 0.3 mg/g of methanogens with the CSTR and 0.72 mg/g of methanogens with the fixed-bed reactor.

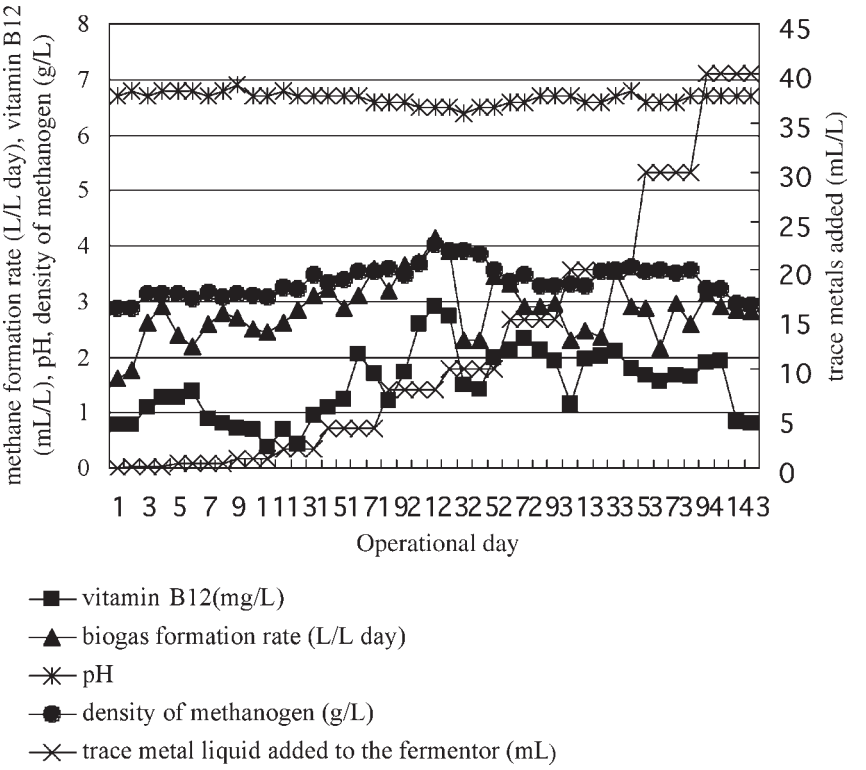

Fig. 3. Course of vitamin B_{12} production, methane production, and methanogen density with different concentrations of trace metals ion added using rock wool fixed-bed reactor.

Krzycki and Zeikus *(11)* reported that the highest corrinoid level of 5.6 mg/L was obtained from *Methanosarcina bakeri* strain MS using MeOH as substrate. The results presented herein show that alcohol waste fluids utilizing acclimated meth-anogens have a lower level of corrinoids. Methanol may be considered a stimulatory factor, and additional studies are in progress.

The results in the present study show that rock wool is effective at improving the performance of the fermentor. It could be possible that, owing to the rock wool, the fixed-bed has a higher porosity, and the methanogens immobilized on the rock wool support system have the potential to offer a high methane production rate and high vitamin B_{12} concentration in the culture broth. We checked the rock wool carrier after 42 d of digestion, and although it looked black, it maintained its original shape and did not collapse during the long digestion period.

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