mixed with hydrogen medium (HM) in the 250 ml of serum bottle. The ratio of activated sludge: media = 1:9 (v/v). Existing air in the bottle is removed by way of flushing using nitrogen gas. The enrichment culture was incubated in a shaker incubator at 65 ° C for 72 hours (C1 culture), 10% of the fermentation broth was subcultured to sterile HM medium (C2 culture). The subculturing was done three times. Hydrogen production was analyzed on C2, C3 and C4 cultures, whereas the metabolite (organic acids) and reducing sugar were analyzed on C4 culture. The C4 culture was then serially diluted in the same medium solidified by 1,5% Agar and purged with nitrogen gas to create anaerobic condition. The cultures were incubated for 72 hours. Single colonies were isolated, characterized and identification. Hydrogen production was analysed by a gas chromatograph (GC-Shimadzu). Organics acid and etanol productions were analyzed by HPLC, whereas reducing sugar was analysis by DNS method.

Identification of bacterial isolates.

Identification was based on sequences of genes encoding 16S rRNA. 16S rRNA gene was amplified by PCR from genomic DNA using the forward primer 27f with the sequence (5' - AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1492r with the sequence (5' - TACGGHTACCTTGTTACGACTT-3'). PCR reactions were performed using the KIT (Pure TaqTMReady - To GoTMPCR Beads Amersham Biosciences) . The PCR program used was as follows : 95 ° C for 1 min , 30 cycles (95 ° C for 1 min , 50 ° C for 1 min and 72oC for 1.5 min) and 72oC for 10 minutes to an extension of the final product . PCR products were extracted from the gel and purified using a DNA gel extraction KIT. Purified DNA fragments used as templates for sequence analysis (sequencing). Partial DNA sequence of the 16S rDNA of the isolates were identified by entering the data analyzed DNA sequences into BLAST program at http://www.ncbi.nlm.nih.gov/BLAST/, sites used to obtain DNA sequence comparison is most similar to the data available in GenBank .

RESULTS AND DISCUSSION

Hydrogen production of enrichment culture

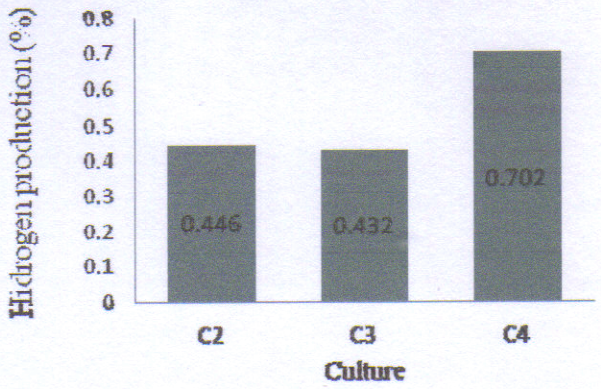

Figure 1. Hydrogen production from enrichment cultures