

Figure 1 shows the H₂ production of the enrichment culture C2, C3 and C4. This suggests that there are H₂ production bacteria in the culture. Among the three enrichment culture, the C4 culture had the highest production of H₂, because sub culturing process resulted in the selection of the bacteria. Regardless of the subculturing conditions, the microbial communities of the the C4 culture were dominated by H₂ production bacteria. The production of H₂ by the C4 culture was 0.702%, while the H₂ production by the C3 culture and C2 culture was 0.432% and 0.446%, respectively.

Table 2 summarizes the acid and ethanol production, sugar utilization and pH of the C4 culture. The metabolite that were detected were acetic acid, propionic acid and ethanol.

Tabel 2. Metabolite production and pH of the C4 culture

| Metabolite/reducing sugar/pH | Periode incubation (hour) | |
|------------------------------|---------------------------|--------|
| | 36 | 72 |
| Acetic acid | 7.73 | 24.970 |
| Propionic acid | 9.89 | 0.000 |
| Ethanol | 0.02 | 0.002 |
| Reducing sugar | 0.27 | 0.067 |
| pH | | 5.500 |

Simultaneously, hydrogen gas was formed during a decomposition of sugar into low molecular weight organic acids such as acetate, butyrate, propionate, etc. Oh et al. (2003), also showed that during the process of the hydrogen production, the metabolites such as acetic acid, propionic acid and ethanol were formed, while the O-thong et al. (2011) found that the dominant metabolites formed are acetic acid, butyric acid and ethanol. In the same condition microbes also produce ethanol via different pathway (Koskinen, 2008). Some bacteria use Entner–Mayerhof phosphoroclastic pathway in the oxidation of substrates, which results in the production of ethanol and hydrogen along with organic acids (e.g., acetate, butyrate, and lactate). As presented in Table 1, during periode incubation, reducing sugar is degraded into organic acids, ethanol and hydrogen. Reducing sugar decreased, while acetic acid acid increased from 7.73 mmol to 24.97 mmol. Acetic acid is the major metabolite.

Isolation, identification, and metabolite characterization.

From the C4 culture, five bacterial isolates have been obtained. Based on the examination on the similarities of the morphology of isolates obtained, three isolates have been selected. They were BYM1, BYM2, and BYM3. Each isolate had a circular colony, opaque and cream colored, long rod-shaped cells (approximately 1x3um), gram-positive and facultatively anaerobic. According to the sequence of their 16S-rDNA, the BYM1, BYM2, and BYM3 were identified as *Bacillus circulans* with 97% similarities. The three isolate bacteria were then designated as *Bacillus circulans* BYM1, *Bacillus circulans* BYM2 and *Bacillus circulans* BYM 3.

Bacillus circulans is gram positive, occasionally curved rods, colonies are opaque, cream colored, slightly convex, growth on nutrient agar is thin and facultatively anaerobic. *B. circulans* is a typical chemoorganoheterotrophic bacterium using mono-, di- and polysaccharides and polyhydroxylic alcohols as sources of carbon, energy and