electrons. The activity of *B. Circulans* is attributed to its metabolites and their specific reactions such as acidolysis, alkalysis and complexolysis (*4*). *B circulans* are hydrolysisi of strach, acid production from such sugar, i.e. glucose, fructose, galactoses, cellobiose, and sucrose. Spore are numerous in soil, may be isolated from sewage. (S.N. Groudev, Use of heterotrophic microorganisms in mineral biotechnology, *Acta Biotechnol. 7* (1987) 299–306. ABIS encyclopedia, www.tgw1916.net/Bacillus/circulans.html. There is only a few reports on H2 producing processes with *Bacillus circulans.*

The ability of isolate and combination of the isolates to produce hydrogen was examined in HM medium. Hydrogen and metabolite production of isolate and their combination are summarized in Table 3. The dominated metabolite by-products was acetic acid. The acid production by the isolate and their combination culture were higher than the C4 culture. The acid production could reflect the isolate ability which has considerable effect on the hydrogen production. Given this point, the hydrogen production by the mixed culture BYW2-BYW3 was higher than the C4 culture. The hydrogen production by mixed culture BYW2-BYW3 was 0,75%, while H2 production by C4 culture was 0.70%. Because the acid production by isolate and their combination was higher, the cultures pH become lower. The hydrogen was detected only in mixed BYW2-BYW3 culture. This may be due to poor implementation of research resulting in gas leakage, because ssimultaneously the gas will be formed during the formation of organic acids.

Tabel 3. pH, H_2 and metabolite production of each isolate bacteria and their combination

Isolate and their combination	H_2 production (%)	Metabolites (mmol)		pH
		Acetic Acid	Propionic acid	
BYW1	0	75.9	0,68	4,53
BYW2	0	117.9	0,45	4,67
BYW3	0	128.6	2,15	4,55
BYW1-BYW2	0	139.0	11,94	4,50
BYW1-BYW3	0	111,0	0,77	4,63
BYW2-BYW3	0,75	123.3	1,30	5,14
BYW1-BYW2-ByYW3	0	123.8	0,77	4,61

As has been noted, hydrogen gas generated by C4 culture or mixed culture of BYW2-BYW3 were relative low. In order to induce bacteria for hydrogen production, the following items should be gathered:

1. Fermentation condition are anaerobically
2. Intensive biogas separation to retrieve hydrogen during the process. When the hydrogen production increases the reaction becomes thermodynamically unfavorable because of the increase in H_2 partial pressure (Nath and Das, 2004)
3. Adding buffer, because the medium pH usually decreases drastically due to the accumulation of organic acids as fermentation proceeds and a lower pH suppresses H_2 production significantly (Oh et al., 2003).
4. Mixed the culture with a hydrogen-production photosyntetic bacteria. Microbial H_2 production can be either photosynthetic or non-photosynthetic. In dark fermentation (non-photosynthetic), carbohydrate substrates are decomposed into