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kill vegetative cells. The spore forming microorganisms then conditioned in anaerobic conditions by bubbling nitrogen into the bottle containing the growing medium. After anaerobic condition, further incubation for 72 h at 60 ° C for 72 hours continuously to obtain the initial culture or sub-culture I (SKI). Isolation process followed by acclimatization process with the aim to obtain a stable microbial means if microbes were grown in the same media will produce the same metabolic products. Acclimatization performed three times with acclimatization time is 72 hours. Acclimatization is done by rejuvenating microbes in the new media with the same treatment. Microbial isolates named sub-culture I (SC I). Isolates were already acclimatized called sub-culture II, III and IV respectively. Sub culture IV is stable microbial. For more details, isolation, acclimatization and production of hydrogen and acetic acid using SK IV with different substrate can be seen in the flow chart below (Figure 1).