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## Biological systems for simultaneous methanation of CO<sub>2</sub> and H<sub>2</sub> by anaerobic microorganisms

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Biogas upgrading is an emerging technology for fuel production that can be used in transportation sector or substitute natural gas. Nowadays, the biological route for biogas upgrading via hydrogen assisted methanogenesis is gaining increased attention. The success of this process is strongly dependent on the applied environmental parameters (e.g. hydrogen partial pressure) and their subsequent influence on the microbial consortium (e.g. efficiency of syntrophic interactions). This abstract provides a collective synopsis of our previous studies on development of systems for biological upgrading of biogas with H<sub>2</sub>. Two different concepts (in-situ and ex-situ biogas upgrading) were developed. For in-situ biogas upgrading, hydrogen was directly injected into the primary or secondary biogas reactor, and hydrogen could be converted to CH<sub>4</sub> together with CO<sub>2</sub> by autochthonous hydrogenotrophic methanogens. It was shown that after the H<sub>2</sub> addition, the CH<sub>4</sub> rate increased by 45%, resulting in an average CH<sub>4</sub> content of approximately 85%, with a maximum of 94%. The increase of the pH to 8.5, due to the CO<sub>2</sub> conversion, was not an inhibitory factor, demonstrating the adaptation of microorganisms to these pH levels. The profiles of the microbial communities prior and after the H<sub>2</sub> addition showed distinct differences. Changes in the archaeal community and more specifically increase in the relative abundance of *Methanobrevibacter* sp. and *Methanoculleus* sp. indicated that the methanogenic pathway was clearly shifted from acetoclastic to hydrogenotrophic. For ex-situ biogas upgrading, hydrogen and biogas were together injected into an anaerobic reactor containing enriched cultures that can convert H<sub>2</sub> and CO<sub>2</sub> to CH<sub>4</sub>. Results demonstrated that the reactors were able to convert efficiently the injected H<sub>2</sub> and CO<sub>2</sub> to methane. More specifically, the final output content of CH<sub>4</sub> reached 96% at a hydrogen loading rate of 3.6 L/L<sub>reactor</sub>·d H<sub>2</sub>. During stable operational conditions, samples were collected from all reactors for microbial analysis based on high throughput 16S rRNA amplicon sequencing. The massive increase of hydrogenotrophic methanogens, such as *Methanothermobacter thermautotrophicus*, and syntrophic bacteria demonstrates the selection-effect of H<sub>2</sub> on community composition.