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Publisher's version / Version de l'éditeur:

*Proceedings of the Pacific Rim Summit on Industrial Biotechnology and
Bioenergy, 2013-12-11*

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Characterization of an adapted microbial population to the bioconversion of carbon monoxide into butanol using next-generation sequencing technology.

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Background

Biobutanol is increasingly regarded as the second generation biofuel of choice. Microbial production of butanol is however still conventionally based on the utilization of carbohydrates as carbon feedstock. More and more companies are interested in using alternative carbon sources, such as biomass, notably by combining conversion of biomass into syngas via gasification and microbial fermentation of syngas components.

To date, only few syngas-fermenting microorganisms that can produce butanol are known. Discovering new microorganisms or microbial consortia capable of fermenting syngas into liquid biofuels, and engineering them to make them commercially attractive is thus primordial in a strategy to develop an economically viable platform for biobutanol production. This presentation will discuss the use of next-generation sequencing technology to perform microbial community analyses of anaerobic undefined mixed cultures, with the objective to identify microbial species particularly adapted to the bioconversion of carbon monoxide (CO), a major component of syngas, into butanol.

Methods

CO enrichment experiments were performed with an anaerobic granular sludge treating agricultural wastes. The sludge was incubated during two months at mesophilic temperature (35°C), with continuous CO injections in the headspace, creating an atmosphere of 100% CO.

Liquid samples were collected after one and two months of operation, for subsequent microbial community analyses. Presence of volatile fatty acids (VFA) and alcohols (particularly butanol) was verified by gas chromatography after two months.

DNA was extracted from the initial sludge and from the samples collected during enrichment experiments. Bacterial and archaeal 16S rRNA genes were amplified and then sequenced using the Ion Torrent sequencing technology. Data generated were analyzed using the ribosomal database project (RDP) Classifier web tool.

Results

Notable differences were observed in the microbial community structure between the initial anaerobic microbial consortium and the CO-adapted population obtained after enrichment experiments. Diversity of the bacterial population notably decreased after CO enrichments, with 17 different phyla identified in the initial sludge, as opposed to 14 after one month and 11 after two months of operation. *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*, which were the three dominant phyla in the initial sludge, significantly decreased after CO enrichments. On the opposite, *Firmicutes* became ultra dominant, representing more than 82% of the total bacterial sequences after two months. The order *Clostridiales* became the most important in number, with *Acetobacterium* being the most representative genus (49.8%).

Archaeal diversity was less important since almost all the sequences were classified into the same phylum, *Euryarchaeota*, for both the initial anaerobic microbial consortium and the CO-adapted population. After CO enrichments, the class *Methanobacteria* increased, with *Methanobacterium* becoming the most representative

archaeal genus (44.6% of the total archaeal sequences). On the opposite, the class *Methanomicrobia*, initially dominant, decreased.

Conclusion and perspectives

The Ion Torrent sequencing technology allowed us to monitor the evolution of the microbial population during CO enrichments, and gave insights on microbial species particularly adapted to the bioconversion of CO into butanol.

Future work will now include the use of known bacterial solvent producers to bio-augment the adapted consortium, to improve its butanol production from CO and optimize and stabilize the performance of the process.