Cross-impact of initial sugars type and microbial community origin and history on fermentative production of biohydrogen and biomolecules from lignocellulosic biomass

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Introduction

Amongst the available technologies to produce green energy, biological processes are considered as the most environmental friendly processes in comparison with the other physico-chemical-based technics. In particular, dark fermentation (DF) is a biological process involving complex microbial consortia which can degrade various complex waste to produce bioenergy (bioH_2) and biomolecules of industrial interest. DF can be applied to lignocellulosic biomass including agricultural residues and food waste. During the dark fermentation process, the biomass is converted into several chemicals: organic acids (eg. acetate, butyrate), solvents (eg. ethanol), and hydrogen. Previous works reported the possibility of producing H_2 from many different types of organic biomass [1]. Although the H_2 yields mainly depend on the total content in readily accessible sugars, the overall conversion performances of the biomass into biomolecules are more related to the nature of the substrate as well as the structure of the degrading microbial community [2]. So far, the relationship between the microbial community characteristics and the type of sugars composing the organic biomass has been only poorly investigated.

The aim of this work is to give new insights on how the different sugar fractions of lignocellulosic biomass (in mono or polymeric forms) can impact the fermentation process in relation with the initial microbial community structure. For that, different origins of inoculum and as storage histories of the same microbial inoculum were investigated.

Material and methods

Batch fermentation tests of glucose, cellobiose, semi-amorphous cellulose, microcrystalline cellulose, arabinose, xylose, and xylan were conducted in four replicates and with two inocula (fresh microorganisms extracted from manure and the same inoculum after a storing period of two months at 35°C). Three additional batch experiments were performed on glucose with fresh aerobic sludge just extracted from Narbonne Waste Water Treatment Plant (France), another one after a storing period at 35°C and the third one freshly taken in the tank after the settling process. Batch tests were performed in plasma bottles of 500 ml, with a working volume of 400 ml, at pH 6, and a substrate concentration of 5 gCOD.l^{-1}, a substrate / inoculum ratio (S/X) of 10, in a minimal medium composed of essential micro-elements. Inocula were heat pre-treated just before experiments during 30 min at 90°C. Anaerobic conditions were set by flushing the headspace with nitrogen gas and the reactors were incubated at 37°C. Gas production was measured by auto-sampling micro-gas chromatograph (µGC) each 8 hours, and metabolites in the liquid phase were analysed at the beginning of the experiment and at the end by High Performance Liquid Chromatography (HPLC). The structure of the initial and final microbial communities was explored by fingerprint SSCP analysis (Single Strand Conformation Polymorphism) and pyrosequencing.

Effect of sugar types on fermentative metabolism

Experiments performed with fresh manure showed that the fermentative metabolite patterns were directly linked to the type of sugars and more precisely to the six or five carbons carbohydrates (C6 or C5). When using the same initial microbial community, metabolic pathways turned to lactate production with the C6 sugars, ie. glucose, cellobiose and cellulose, and were more diverse with arabinose, xylose and xylan (C5), with the production of butyrate, acetate, ethanol and lactate. Because the lactic acid pathway does not generate H_2, ideal substrates for producing H_2 were the C5-based compounds. Furthermore, the use of polymerized substrate lowered substantially the total conversion of the substrate into biomolecules and H_2, which is consistent with reported data in literature [1].

Interestingly, after having stored the inoculum, differences in metabolite distribution between C6 and C5 sugars tended to disappear. In this case, the metabolites produced were the same independently of the type of sugar. Acetate was the main metabolite followed by butyrate and ethanol, which led to higher hydrogen yields with C6. A slight decrease of the H_2 yields was observed with C5 sugars, when compared to the fresh inoculum.

Microbial community analysis by SSCP fingerprinting showed that the initial microbial population structure of each inoculum, fresh or after storage, was quite similar with only small differences. Consistently with the metabolites

distribution patterns, the community structure evolved differently after fermentation suggesting that only the initial minor differences found in the microbial inoculum impacted the overall performances of the fermentation process.

Impact of various microbial structures on the fermentation of glucose

As previously shown, fermentation of glucose by inocula having different storage histories led to different metabolic pathways, ie. mainly lactate from fresh manure and a higher variability with stored manure. The use of aerobic sludge as inoculum led to other metabolic pathways, even more efficient to produce H_2. In this case the main metabolite was butyrate with lower amount of acetate. Accordingly, Guo *et al.* (2014) showed that the butyrate pathway is the metabolic route that should be favoured when producing hydrogen from organic waste [3]. A storage period on this inoculum increased the variability of the metabolic patterns, with the consequence in increasing the production of propionate and a subsequent decrease of the H_2 yields. Principal Component Analysis (PCA) was performed on metabolites produced by each inoculum and showed distinct correlation groups of microbial communities linked to metabolic behaviours (Figure 1).

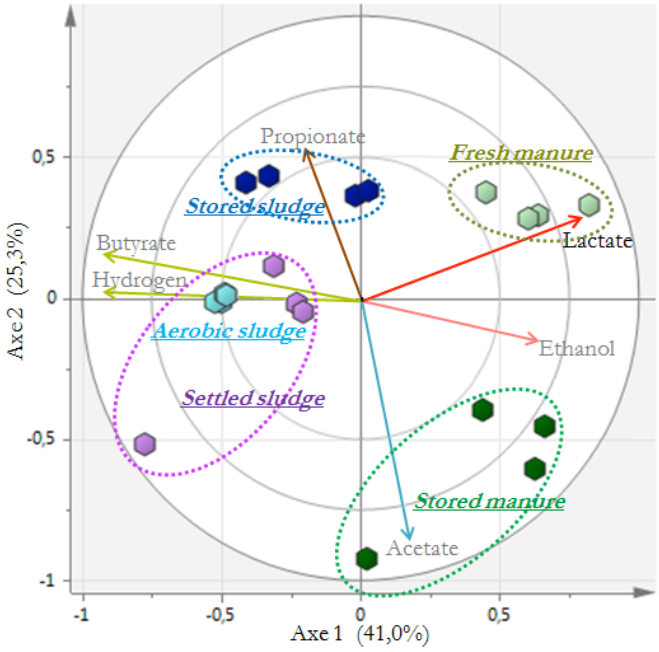

Figure 23. PCA representing the main metabolites produced by the different inoculum after glucose fermentation

According to the microbial SSCP fingerprints, a strong correlation was observed between the diversity in metabolites found after fermentation and the final microbial community structure. Therefore, it was concluded that the metabolic distribution patterns mainly depended on the emergence of a particular microbial community structure composed of several specific species issued from the initial inoculum, and not only on individual species.

Conclusion

Results of this study showed that over the type of substrate that could be used for fermentation, the structure of the microbial community had a significant importance on the final metabolic patterns. Very small differences in the initial microbial composition can impact substantially the fermentation process and subsequent hydrogen yields.

New Generation Sequencing (NGS) analysis of the initial microbial community is under progress and will give new insights on what are the initial and final key species/population structures that were responsible of the drastic changes in the metabolic patterns. This will give new, unique and useful information to further characterize the microbial inoculum which is currently one of the main challenges of H_2 and biomolecules production by dark fermentation.

Keywords

Biohydrogen; C6-C5 sugars; Dark fermentation; Mixed cultures

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