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# IMPORTANCE OF INDIGENOUS BACTERIA IN DARK FERMENTATION OF ORGANIC MATERIALS

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## 1. Keywords

Biohydrogen, Dark fermentation, Thermal pretreatment, Inoculum

## 2. Highlights

- 7 substrates were used to produce H<sub>2</sub> with and with no external microbial inoculum
- H<sub>2</sub> & metabolite productions were equal for indigenous and treated exogenous bacteria
- Thermal pretreatment did not improve H<sub>2</sub> production when no inoculum was added

## 3. Purpose

Dark fermentation (DF) of complex organic matter, such as waste and effluents, can naturally take place with indigenous bacteria or by adding exogenous bacteria from natural environments. Indeed, both contain hydrogen producing bacteria (HPB). Inoculum pretreatment is essential to specifically select spore-forming HPB and to improve H<sub>2</sub> yields. The substrate can also be pretreated to release more soluble sugars (which are proportional to H<sub>2</sub> production)[1]. The main objective of this work was to determine whether external inoculation associated to thermal pretreatment (90°C-15min), conditions often used for inoculum pretreatment[2], could impact the DF performances, i.e. H<sub>2</sub> and metabolite productions, with different organic substrates.

## 4. Materials and methods

Biochemical Hydrogen Potential (BHP) tests were carried out on seven different organic substrates: dates, corn silage, sorghum, microalgae, sewage sludge, OFMSW and food waste (FW). The last two substrates were freshly prepared according to the average composition found in France [3, 4]. Four different kinds of BHP tests were performed. A first one with untreated-substrate with no inoculum (noP-nol), a second one with the substrate pretreated at 90°C for 15 min with no inoculum (P-nol). The third one was performed with no pretreatment and with inoculum (noP-I). For the last one, substrate and inoculum were pretreated simultaneously (P-I). BHP, H<sub>2</sub> and metabolite (volatile fatty acid [VFA], ethanol, succinate, lactate, glycerol, formate and H<sub>2</sub>) measurements were performed, according to Chatellard et al.[5]. For inoculated BHP, the inoculum originated from a wastewater treatment plant, was freeze-dried and stored in a closed box at room temperature.

## 5. Results and discussion

For experiments “noP-nol” and “P-I”, no statistical difference was observed in H<sub>2</sub> and metabolite productions for all substrates (excepted sludge for metabolites), suggesting that indigenous bacteria are at least as effective as exogenous bacteria added after thermal pretreatment (see Figures 1&2). This result is not consistent with Favaro et al. study[6]. Indeed, the authors reported better H<sub>2</sub> production for exogenous pretreated bacteria compared to indigenous bacteria (with OFMSW as substrate). However, OFMSW composition, preparation and probably indigenous bacteria composition were different from the current study. For experiments “noP-nol” and “P-nol”, no positive impact of substrate-pretreatment on H<sub>2</sub> yields was shown for all substrates, excepted with microalgae and sludge, suggesting that thermal pretreatment was not efficient in releasing soluble

sugars[1] and in selecting specifically HPB. Interestingly, inoculation with no pretreatment was detrimental to H<sub>2</sub> production (excepted corn silage and sorghum) but not systematically to metabolite production, e.g., corn silage (experiments “noP-I” and “P-I”). Furthermore, the increase in H<sub>2</sub> production seems to be substrate-dependent, suggesting that microbial communities were selected differently according to substrate structure (carbohydrates)[5] or initial microbial communities. More information on microbial community composition will be presented at the conference.

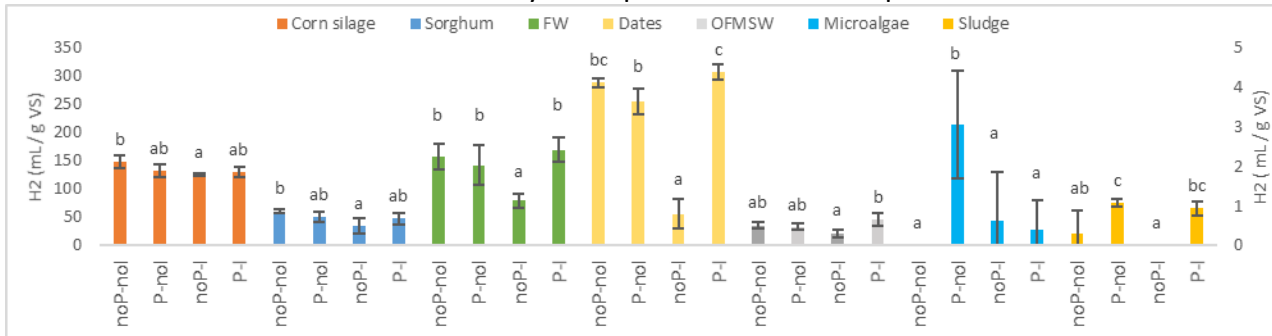


Figure 1: Maximum cumulative H<sub>2</sub> production for all substrates (average). Letters show Tukey test-results (for a same substrate). Microalgae and sludge are represented on the secondary axis

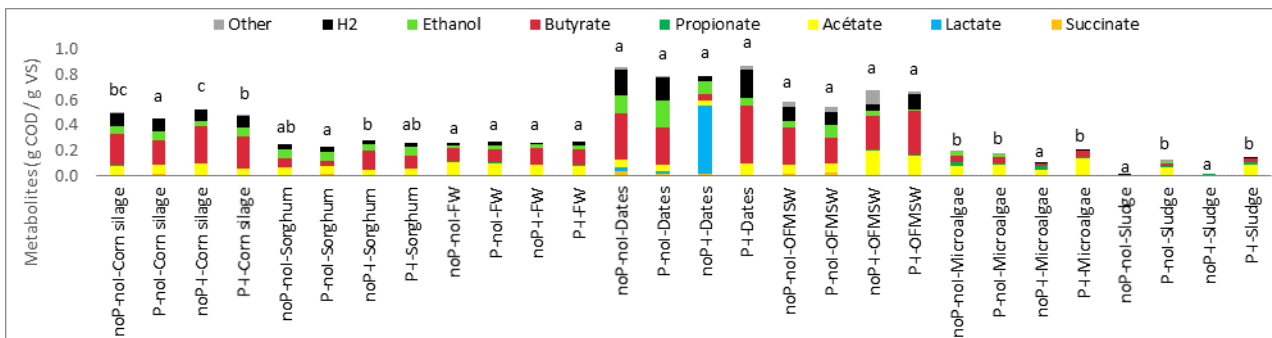


Figure 2: Sum of metabolite production for all substrates (average). Letters show Tukey test-results (for a same substrate)

## 6. Conclusions and perspectives

This study showed that for most substrates, indigenous bacteria seemed to be as effective as external inoculum selected by thermal pretreatment for H<sub>2</sub> and metabolite productions. This suggests that no inoculum nor pretreatment are required to achieve satisfactory DF performances. Further investigations are needed to select HPB among indigenous bacteria by other pretreatments or conditions to improve DF performances.

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