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► **To cite this version:**

K Dauptain, H Carrere, E Trably. Impact of indigenous bacteria inactivation on dark fermentation performances. 9th International Conference on Engineering for Waste and Biomass Valorisation, Jun 2022, Copenhagen, Denmark. hal-04124208

**HAL Id: hal-04124208**

**<https://hal.science/hal-04124208>**

Submitted on 15 Jun 2023

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# IMPACT OF INDIGENOUS BACTERIA INACTIVATION ON DARK FERMENTATION PERFORMANCES

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## 1. Keywords

Biohydrogen, Dark fermentation, Gamma irradiation, Waste

## 2. Highlights

- Prior to DF,  $\gamma$ -irradiation is effective to inactivate indigenous bacteria
- For sorghum, H<sub>2</sub> yields were affected after  $\gamma$ -irradiation
- $\gamma$ -irradiation did not impact the H<sub>2</sub> fermentation of OFMSW
- Indigenous bacteria inactivation induced metabolic changes for both substrates

## 3. Purpose

Dark fermentation (DF) of organic residues can be carried out with indigenous bacteria (living on the substrate) or by adding exogenous bacteria. Literature shows that both types of microbial communities are playing a key role in fermentative hydrogen pathways and in overall bioconversion of complex organic matter [1]. However, the exact contribution of indigenous bacteria in DF process operation has been scarcely investigated and remains unclear as indigenous bacteria are still subsisting when an inoculum is added. This study aims to investigate the role of indigenous bacteria on DF performances, on the orientation of metabolic pathways and bacterial selection.

## 4. Materials and methods

Biochemical hydrogen potential (BHP) tests were performed with or without inoculum as described elsewhere [1]. For each experiment performed with non-irradiated or irradiated biomass, two conditions were investigated: a non-inoculated control, marked "NI" and an inoculated BHP (S/X = 12) marked "I". In addition, the substrate was sterilized by gamma irradiation (45 kGy) to inactivate indigenous bacteria without affecting the biomass structure and composition [2]. Several measures were taken to avoid bacterial contaminations during BHP preparation with irradiated biomass as the use of autoclaved glass bottles or the handling under a microbiological safety cabinet. Two different substrates were used, sorghum and the organic fraction of municipal solid waste (OFMSW), whose characteristics are detailed elsewhere [1].

## 5. Results and discussion

Fig. 1 summarizes DF performances of non-irradiated and irradiated substrates. Regarding DF performances, results showed different behaviors for sorghum and the OFMSW, possibly due to the cooking and synthetic nature of the last-mentioned substrate. For the OFMSW, indigenous bacteria inactivation did not influence the H<sub>2</sub> yields or the microbial bioconversion compared to non-irradiated experiments (with or without inoculum). This is not consistent with results from Favaro et al. [3], who noticed a decrease in the H<sub>2</sub> yield after OFMSW sterilization. For sorghum, DF performances were considerably lower after biomass irradiation (decrease from  $45.2 \pm 1.7$  mLH<sub>2</sub>/gVS<sub>ini</sub> to  $17.8 \pm 12.8$  mLH<sub>2</sub>/gVS<sub>ini</sub>), proving the key role of indigenous bacteria, even after inoculum addition.

Nonetheless, as shown in Fig. 2, indigenous bacteria inactivation impacted the final metabolic profile for both substrates due to changes in bacterial community composition (data will be presented

at the conference). Fig. 2 shows a metabolic change from acetate (34.1%, 23.3%) towards butyrate (39.1%, 49.6%) after OFMSW irradiation compared to non-irradiated samples. For inoculated sorghum using the same inoculum, irradiation led to a different metabolic profile with a decrease in butyrate ( $71.0 \pm 4.9 \% / 30.0 \pm 24.7 \%$ ) and hydrogen ( $10.9 \pm 0.4 \% / 5.7 \pm 4.1 \%$ ) proportions towards ethanol ( $9.6 \pm 2.6 \% / 15.0 \pm 3.1 \%$ ) and lactate ( $0 \pm 0 \% / 34.9 \pm 27.7 \%$ ) for non-irradiated and irradiated sorghum, respectively. This shows the key role of indigenous bacteria in the orientation of metabolic pathways regardless of inoculum addition.

Fig. 1 Hydrogen yield and total bioconversion for non-irradiated and irradiated OFMSW or sorghum. S1/2 = set of experiments number 1/2 (experiments performed at the same time with a same inoculum for inoculated experiments).

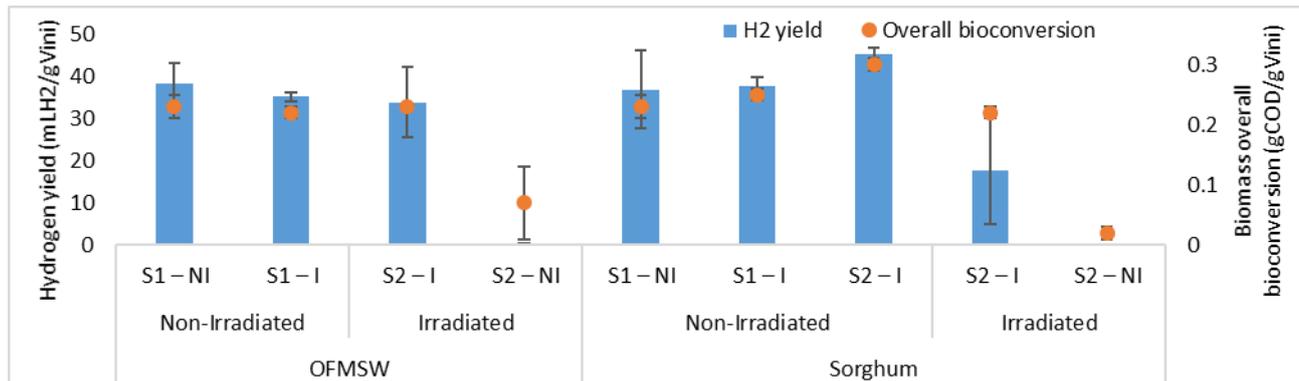
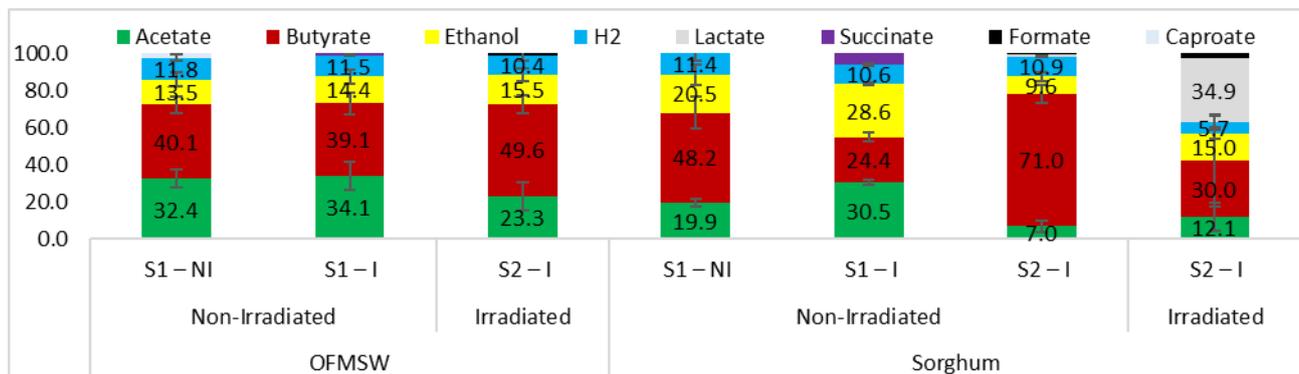


Fig. 2 Metabolite distribution of non-irradiated and irradiated OFMSW and sorghum in percent ( $\text{gCOD/gCOD}_{ini}$ ). NI = no inoculum, I = inoculated, S1/2 = set of experiments number 1/2 (experiments performed at the same time with a same inoculum).



## 6. Conclusions and perspectives

In this study, the key role of indigenous bacteria in obtaining high DF performances was shown, even when an external inoculum is added. Indigenous bacteria inactivation by  $\gamma$ -irradiation was highly detrimental to hydrogen and biomass overall bioconversion for sorghum. In contrast, DF performances of irradiated OFMSW were similar to raw OFMSW. Interestingly, indigenous bacteria inactivation induced metabolic changes from acetate to butyrate, ethanol or lactate, for both substrates. As a consequence, biomass pretreatments impacting indigenous bacteria could negatively influence hydrogen pathways, mitigating the advantages of biomass breakdown after pretreatment.

## 7. References

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