Coupling lactic acid fermentation and dark fermentation of food waste for biohydrogen production

E. Roslan[1,4], J.A. Magdalena[1,5], H. Mohamed[2,4], A. Akhiar[3], A.H. Shamsuddin[2], H. Carrere[1], E. Trably[1]

[1] INRAE, Université de Montpellier, LBE, 102 avenue des Étangs, 11100 Narbonne, France
[2] Institute of Sustainable Energy, Universiti Tenaga Nasional, 43000 Kajang, Selangor, Malaysia
[3] Centre of Excellence for Water Research and Environmental Sustainability Growth (WAREG), Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia
[4] Department of Mechanical Engineering, College of Engineering, Universiti Tenaga Nasional, 43000 Kajang, Selangor, Malaysia
[5]Vicerrectorado de Investigación y Transferencia de la Universidad Complutense de Madrid, 28040 Madrid, Spain

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Presenting author email: eqwan@uniten.edu.my

Food waste (FW) has been investigated as resources for bioenergy such as biohydrogen and biomethane, and other chemical commodities due to its richness in carbohydrates, proteins, and lipids (Sufficiency et al., 2022). Hydrogen production through biological means is a promising route, its lifecycle having low impact to the environment as compared to other methods of hydrogen production (Aydin and Dincer, 2022). FW being easily biodegradable has great potential as substrate for biohydrogen production through dark fermentation (DF). However, being easily biodegradable means organic carbon losses can occur prior its conversion to biohydrogen (Parthiba Karthikeyan et al., 2018). In addressing this issue, lactic acid fermentation (LAF) has long been used as a method to store and preserve food, preparation of silage and recently as part of storage strategy prior to anaerobic digestion for biomethane production (Villa et al., 2020). However, there is scarce information on utilizing LAF as a storage method prior to DF. This might be due to the negative messages surrounding lactic acid bacteria (LAB) being detrimental to hydrogen producing bacteria (HPB). However, a recent review showed that the impact of LAB on DF is deemed inconclusive (García-Depraect et al., 2021).

This study aims to couple LAF as a storage method of FW prior to conversion to biohydrogen through DF. It focuses on the metabolite production during storage and consequently its effect on the second step DF. Microbial communities present at the end of both LAF storage and DF conversion to hydrogen were investigated.

FW utilised in this study was freshly reconstituted representative of FW composition in France as proposed by (Noguer et al., 2022), at 10% total solids (TS). FW was stored for 15 days at 4°C, 10°C, 23°C, 35°C, 45°C and 55°C. Liquid and gas samples were collected periodically and analysed for organic acids, ethanol, and gas composition. After storage, the stored FW underwent DF at pH 6 and dilution to 1%TS to prevent inhibition by metabolites produced during storage. DF bottles were connected to a micro gas chromatograph with automated sampling in every two hours to measure the hydrogen gas produced. Additionally, biomass was sampled and analysed for microbial communities.

After 15 days of storage, the main metabolites accumulated were lactate and ethanol, with smaller quantities of acetate. Storage at 10°C and 23°C favoured ethanol accumulation over lactate whereas at 4°C, 35°C, 45°C, 55°C lactate dominated, as summarized in Table 1.

Table 1: Metabolites accumulated at the end of lactic acid fermentation storage of food waste for 15 days at 4°C, 10°C, 23°C, 35°C, 45°C and 55°C

Storage temperature °C	Metabolite concentration (gCOD/L)			Reaction Advancement [1](%)
	Lactate	Ethanol	Acetate	
4	4.2±0.5	1.1±0.2	1.1±0.1	5.0±0.5
10	6.0±0.2	10.1±3.9	1.1±0.0	12.2±3.0
23	13.1±0.2	22.5±3.7	1.3±0.0	30.3±3.3
35	15.3±2.0	6.3±0.6	1.1±0.1	19.3±2.2
45	6.4±1.0	3.2±0.4	0.6±0.1	8.6±1.0
55	2.2±0.7	-	-	2.0±0.6

Regardless of the different metabolite profile obtained by the end of LAF storage, the conversion of the stored FW showed no difference in maximum biohydrogen production, except for FW stored at 55°C, as shown in Table 2. All other storage temperatures at 4°C, 10°C, 23°C, 35°C, 45°C yielded not statistically different biohydrogen production as compared to the control (fresh FW). All samples stored FW had higher maximum production rate than the control.

[1] Total metabolites and gas accumulated in gCOD/L over the initial amount of biodegradable COD/L of substrate, in percentage.

Table 2: Variable values Pm, Rm and λ from modified Gompertz equation fitting. Superscript letters represent Tukey's test results; values sharing the same letters are not statistically different

Substrate storage temperature (°C)	P_m, maximum production (mL/gVS)	R_m, maximum production rate (mL/gVS·d)	λ, lag phase (d)
Fresh FW	76.8±11.9^{a}	29.3±11.1^{a}	0.8±0.1^{cd}
4	80.0±5.7^{a}	46.1±5.9^{ab}	0.4±0.1^{a}
10	89.3±0.5^{a}	53.4±10.1^{ab}	0.7±0.0^{bc}
23	79.0±5.1^{a}	71.8±17.8^{b}	1.1±0.0^{e}
35	94.0±19.9^{a}	69.5±14.3^{b}	0.5±0.1^{ab}
45	83.4±2.1^{a}	57.8±10.6^{ab}	0.6±0.0^{ab}
55	141.4±34.6^{b}	75.6±20.6^{b}	0.9±0.1^{d}

By the end of LAF storage, LAB *Lactobacillus* sp. was dominant genus in all storage temperatures and in one sample stored at 55°C. By the end of DF, microbial communities became more diverse with the emergence of *Clostridium* sp., a well known HPB, co-existing with LAB previously present during storage.

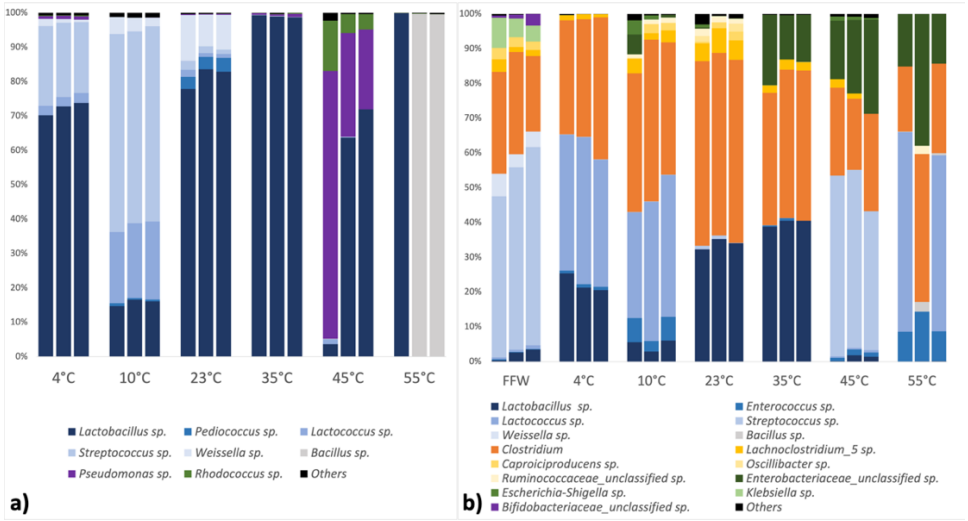

Figure 1: Relative abundance at genera level for a) lactic acid fermentation storage b) dark fermentation

This study showed that temperature affected the metabolites accumulated during LAF storage. Regardless of this difference, it had no impact on the biohydrogen yield. LAF was demonstrated to be an efficient storage method without affecting the biohydrogen potential. Storage at 55°C showed improvement of hydrogen yield by 84%. For all cases, stored FW showed improvement of maximum production rate in DF as compared to control. HPB *Clostridium* sp. emerged in DF reactors for biohydrogen production, even after 15 days of storage in low pH LAF. *Clostridium* sp. and *Lactobacillus* sp. co-existed in DF reactors, showing no negative impact on each other. In conclusion, LAF was shown as efficient storage method of FW with a high flexibility in terms of operating temperatures, making the process usable in a wide range of conditions and countries. This opens new opportunities for managing FW at larger scale.

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