Lactic Acid Fermentation as Storage Method of Food Waste Prior to Dark Fermentation

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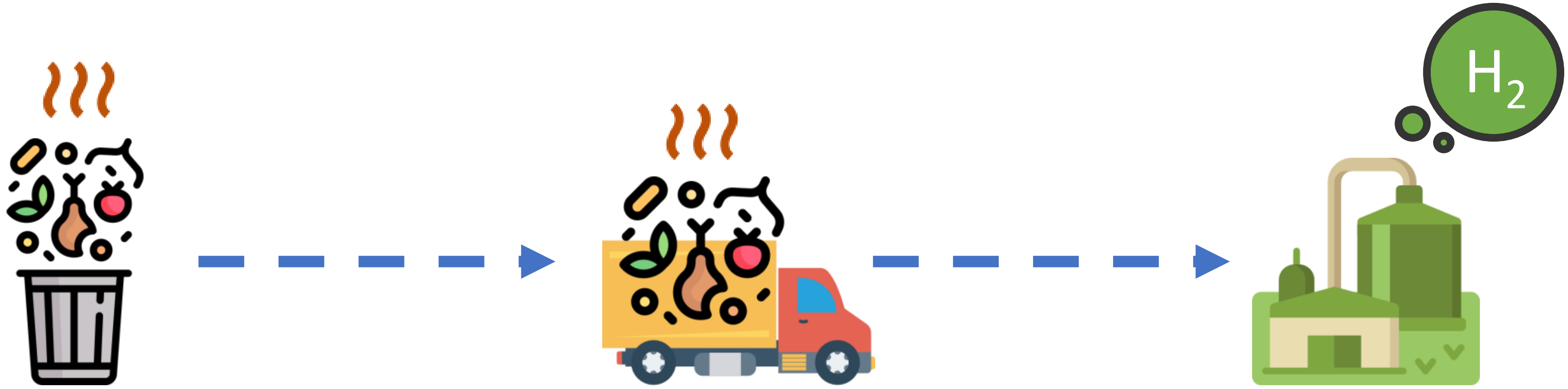
Food waste as Dark Fermentation Substrate



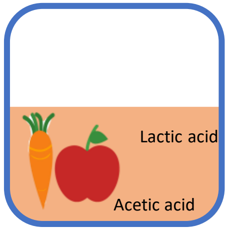
Organic carbon losses during storage / transport [1]
Fermentation can occur during transport [2]

Storage is necessary to preserve the Biohydrogen Potential (BHP) of Food waste (FW)

[1] O. Parthiba Karthikeyan, E. Trably, S. Mehariya, N. Bernet, J. W. C. Wong, and H. Carrere, "Pretreatment of food waste for methane and hydrogen recovery: A review," *Bioresour Technol*, vol. 249, no. July 2017, pp. 1025–1039, 2018, doi: 10.1016/j.biortech.2017.09.105.
[2] A. Noblecourt, G. Christophe, C. Larroche, and P. Fontanille, "Hydrogen production by dark fermentation from pre-fermented depackaging food wastes," *Bioresour Technol*, vol. 247, no. July 2017, pp. 864–870, 2018, doi: 10.1016/j.biortech.2017.09.199.

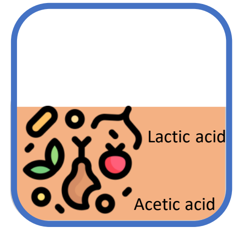
Lactic Acid Fermentation (LAF) as Substrate Storage

LAF for food storage



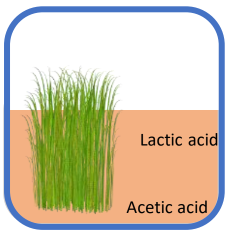
Used to **preserve food** for probably 3000 years [3]

LAF for waste valorization to chemicals



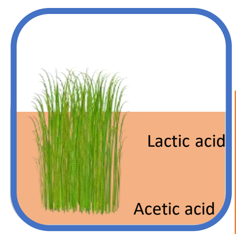
Recently been used to **produce higher value products (chemicals) [4]**.

Ensiling for animal feed

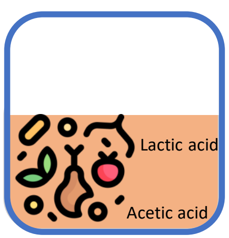


Part of ensiling process, used to **preserve crops /** produce silage for **animal feed**.
Objective: **preserve nutrition [5]**.

Ensiling for biomethane



Recently used as **storage strategy** prior to **anaerobic digestion**.
Objective: **maximize Bio-methane Potential [5]**.



LAF for dark fermentation

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[3] J. M. Wilkinson, K. K. Bolsen, and C. J. Lin, "History of Silage," in *Silage Science and Technology*, 2003. doi: https://doi.org/10.2134/agronmonogr42.c1.
[4] C. Chenebault, R. Moscoviz, E. Trably, R. Escudié, and B. Percheron, "Lactic acid production from food waste using a microbial consortium: Focus on key parameters for process upscaling and fermentation residues valorization," Bioresour Technol, vol. 354, no. April, 2022, doi: 10.1016/j.biortech.2022.127230.
[5] R. Villa, L. Ortega Rodriguez, C. Fenech, and O. C. Anika, "Ensiling for anaerobic digestion: A review of key considerations to maximise methane yields," *Renewable and Sustainable Energy Reviews*, vol. 134, no. September, p. 110401, 2020, doi: 10.1016/j.rser.2020.110401

Lactic Acid in Dark Fermentation

Glucose

Hydrogen producing pathways

HPB (Hydrogen Producing Bacteria)
e.g.: Clostridium butyricum

Most common inhibition for long-term operation

Compete

Produc

"

the

ng

B

Lactic Acid producing p

1) LAB and Lactic acid were commonly assumed to be detrimental to hydrogen production (until recently). There are also positive relationships reported.
2) Lactic Acid can be converted into hydrogen if the right microbial community is present

(to

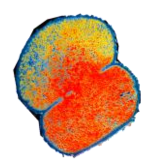
is a

"blindspot" that reduces diversity [9]

LAB (Lac
e.g.: Lactobacillus
Enterococcus

Lactic acid

Lactic Acid to hydrogen pathway



LU-HPB
e.g.: Megasphaera elsdenii

Is not heat-shock tolerant
Able to produce hydrogen from lactate [8]

[6] M. lou Hillion *et al.*, "Co-ensiling as a new technique for long-term storage of agro-industrial waste with low sugar content prior to anaerobic digestion," *Waste Management*, vol. 71, pp. 147–155, 2018, doi: 10.1016/j.wasman.2017.10.024.
[7] O. García-Depraect *et al.*, "A review on the factors influencing biohydrogen production from lactate: The key to unlocking enhanced dark fermentative processes," *Bioresour Technol*, vol. 324, no. December 2020, 2021, doi: 10.1016/j.biortech.2020.124595.
[8] A. Ohnishi, Y. Hasegawa, N. Fujimoto, and M. Suzuki, "Biohydrogen production by mixed culture of Megasphaera elsdenii with lactic acid bacteria as Lactate-driven dark fermentation," *Bioresour Technol*, vol. 343, no. September 2021, p. 126076, 2022, doi: 10.1016/j.biortech.2021.126076.
[9] A. Ohnishi, Y. Hasegawa, S. Abe, Y. Bando, N. Fujimoto, and M. Suzuki, "Hydrogen fermentation using lactate as the sole carbon source: Solution for 'blind spots' in biofuel production," *RSC Adv*, vol. 2, no. 22, pp. 8332–8340, 2012, doi: 10.1039/c2ra20590d.

Materials: Food waste

Mixed based on % in recipe

Blended for 10 minutes total

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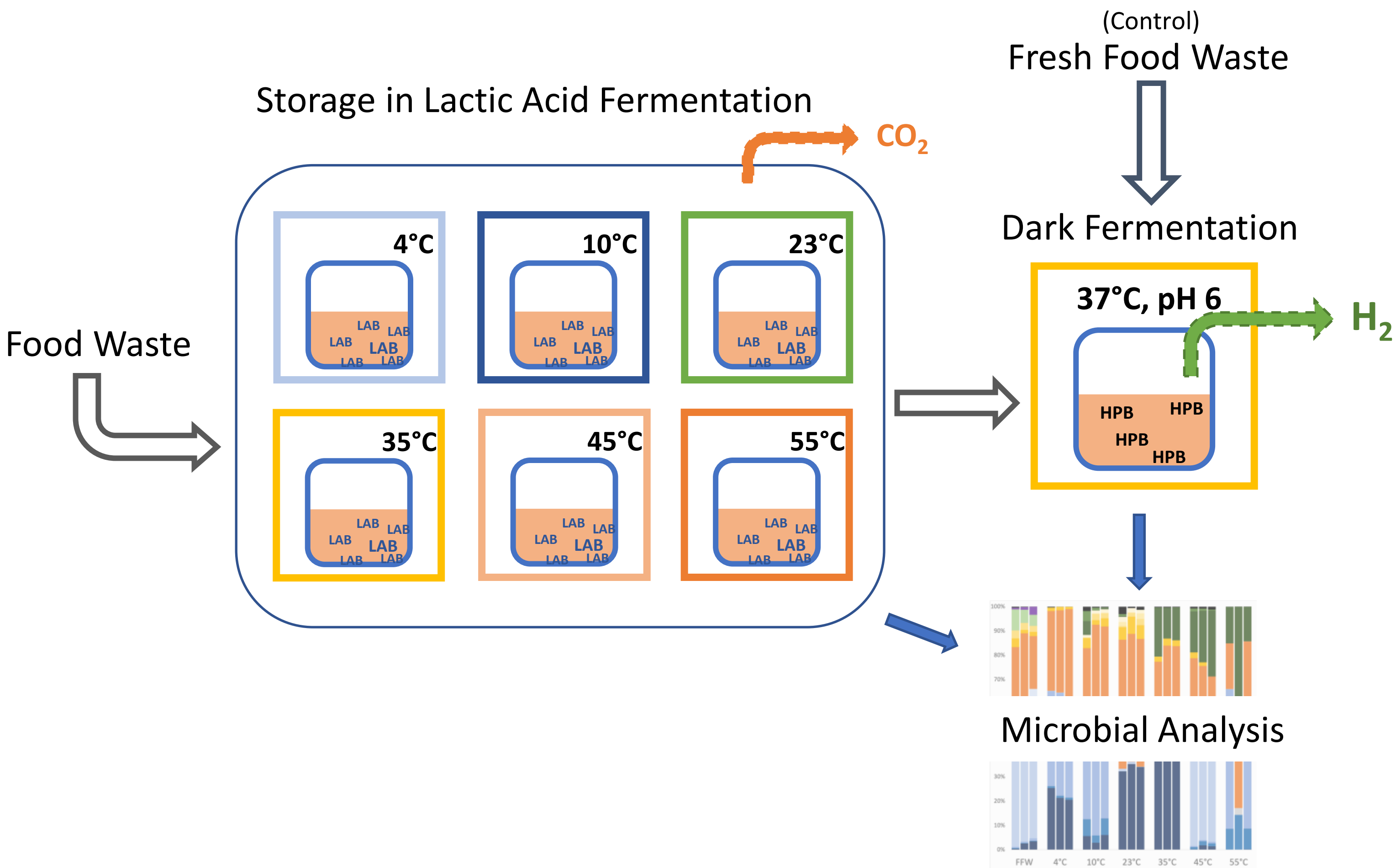
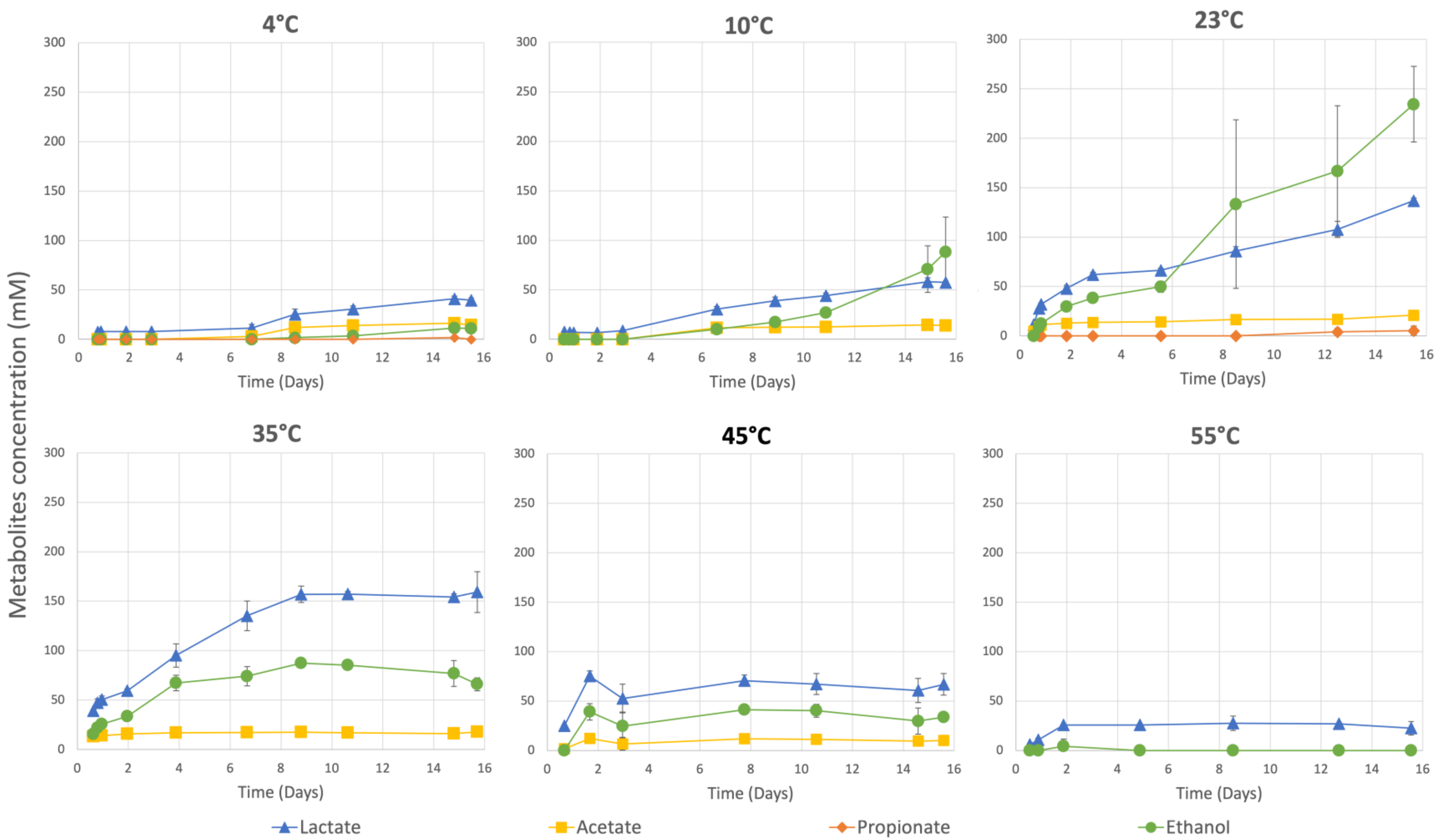
Methods



(Control)
Fresh Food Waste
Storage in Lactic Acid Fermentation
CO_2
Food Waste
4°C
10°C
23°C
35°C
45°C
55°C
LAB
Dark Fermentation
37°C, pH 6
H_2
HPB
Microbial Analysis
FFW
4°C
10°C
23°C
35°C
45°C
55°C

Metabolites production during storage



Homolactic pathway: Glucose → 2Lactate

Heterolactic pathway: Glucose → Lactate + Ethanol + CO_2

Ethanolic pathway: Glucose → 2Ethanol + $2CO_2$

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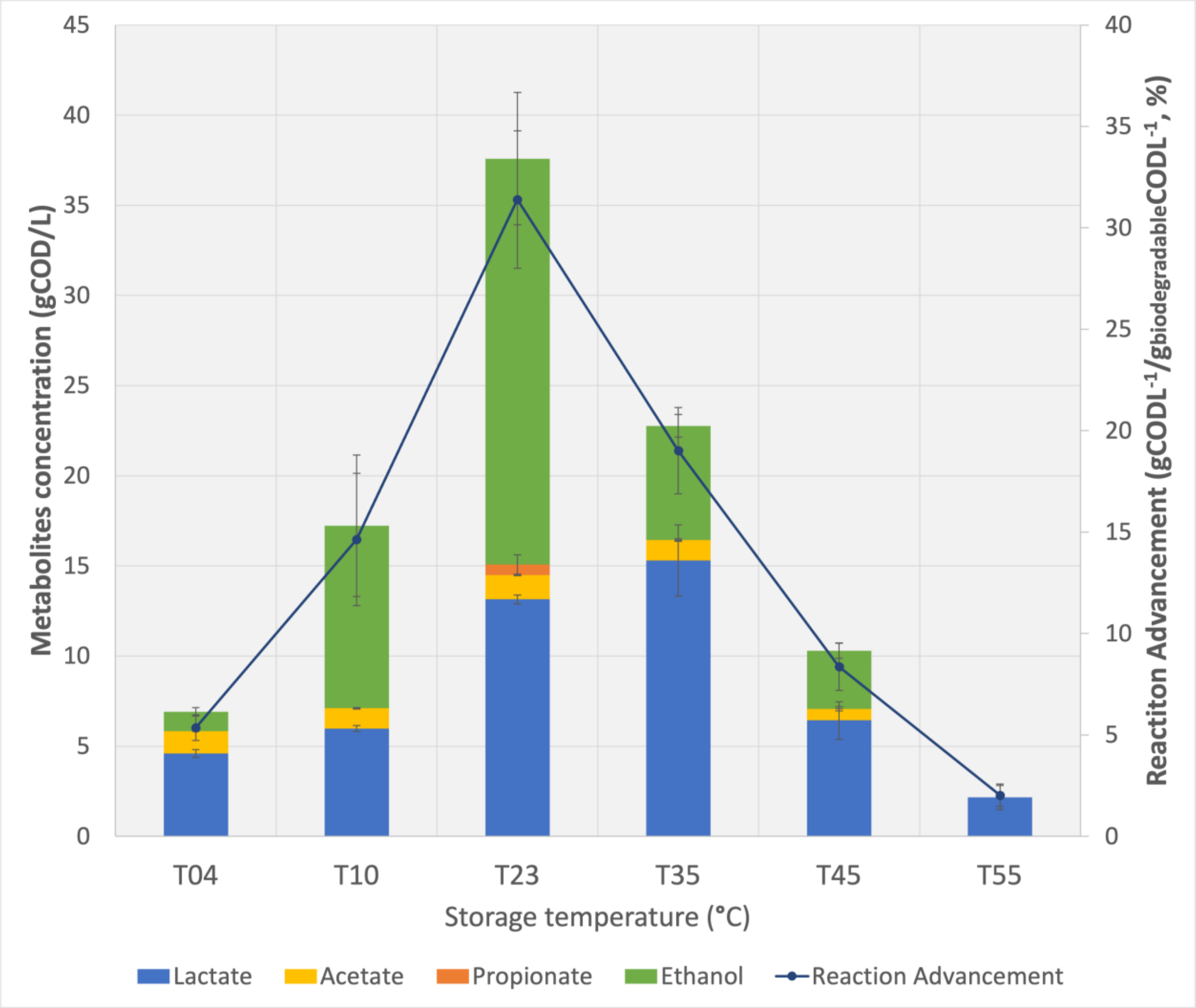
Reaction advancement after storage



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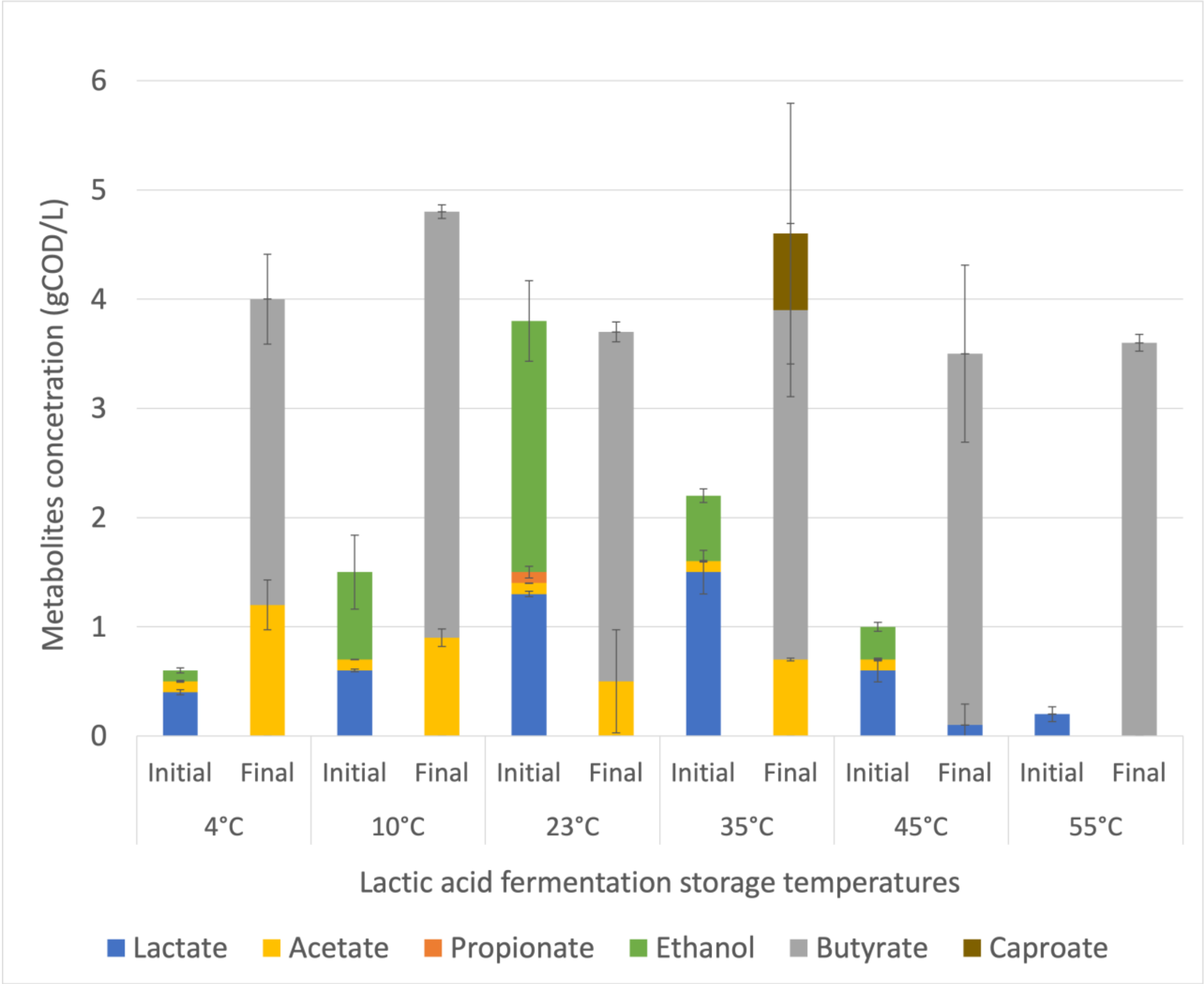
Hydrogen production via dark fermentation

Substrate storage temperature (°C)	P_m, maximum production (mL/gVS)	R_m, maximum production rate (mL/gVS·d)	λ, lag phase (d)
Fresh FW (Control)	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}	
55	141.4 ± 34.6^{b}	75.6 ± 20.6^{b}	

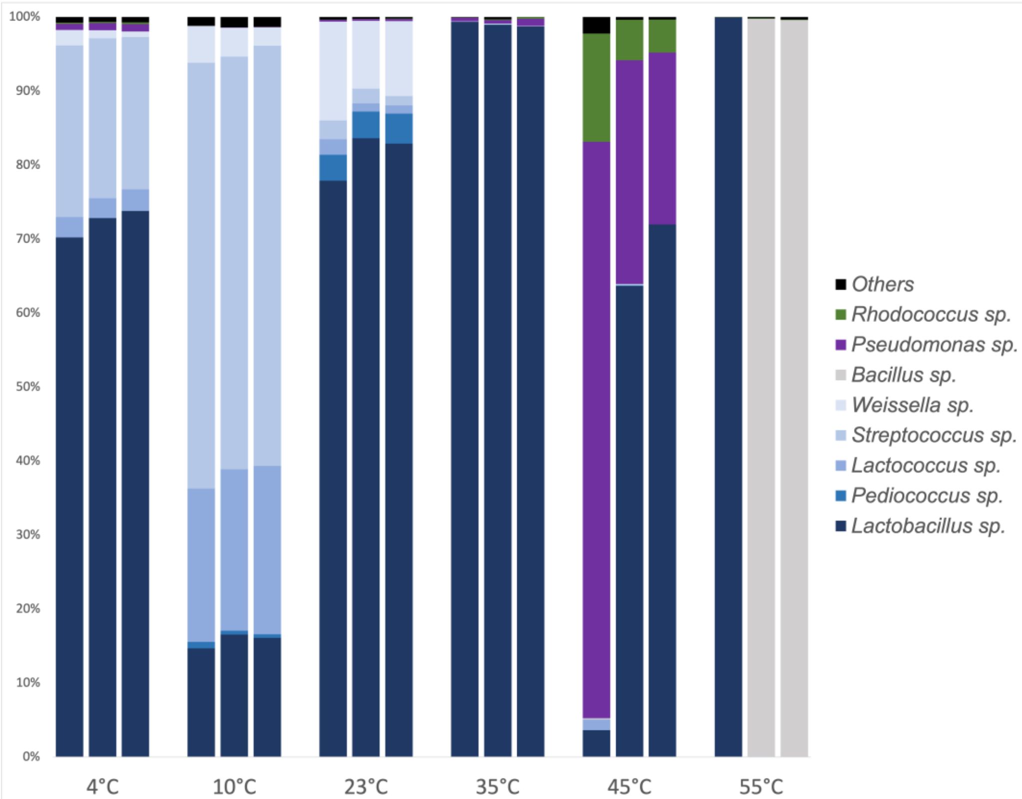
Rm increased by at least 57%

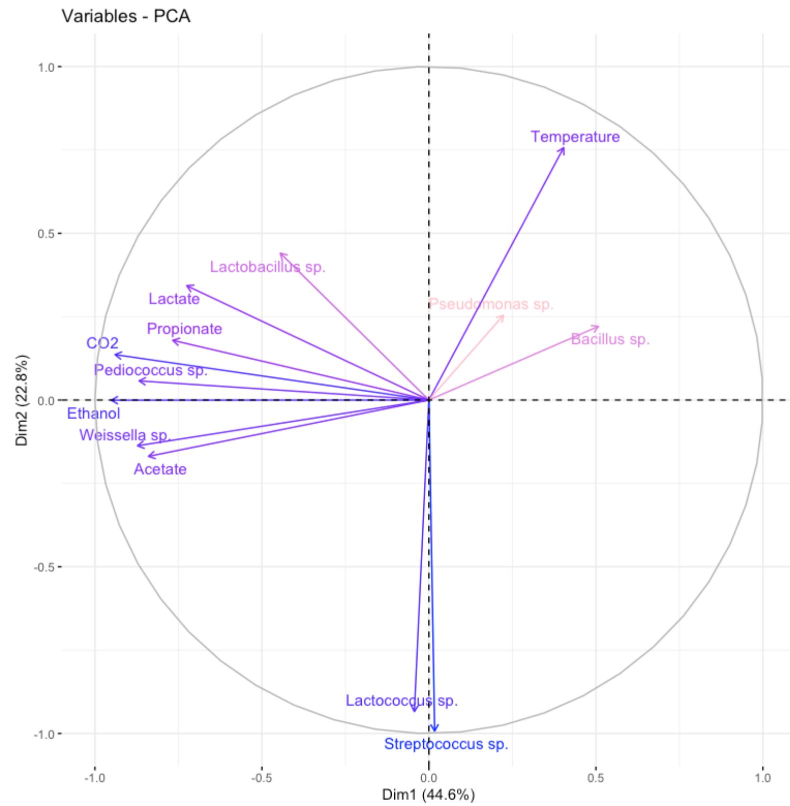
Statistically similar yield except for storage at 55°C (~83% higher)

Initial and final metabolites concentration in dark fermentation



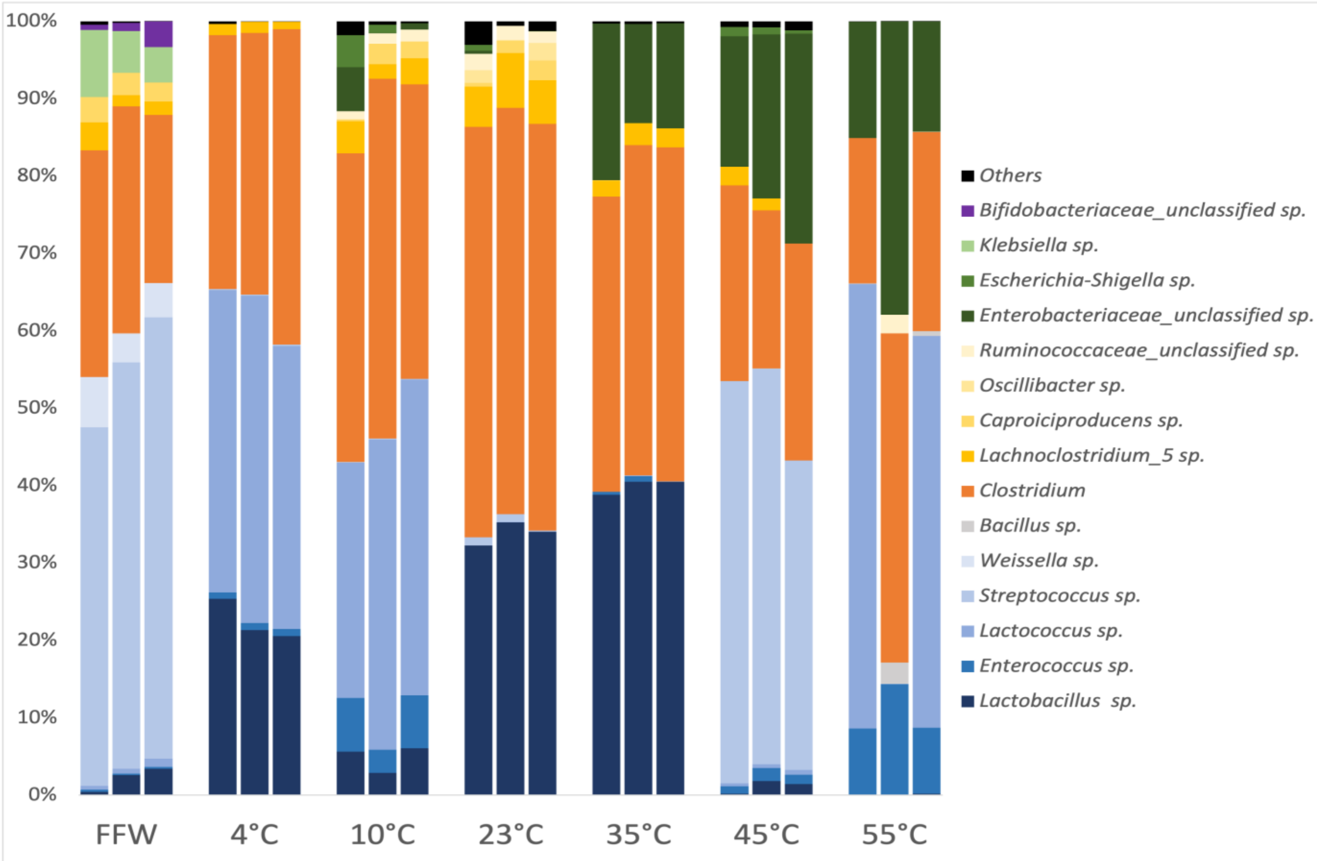
Microbial community present at the end of storage





Lactobacillus sp.: Homolactic or heterolactic (species dependent)
Streptococcus sp.: Homolactic, heterolactic when carbohydrate-restricted
Lactococcus sp.: Homolactic
Weissella sp.: Heterolactic, ethanol with lactate
Pseudomonas: Strictly aerobic, some species can be anaerobic using nitrate
Pediococcus sp.: Homolactic or heterolactic

Microbial community present at the end of dark fermentation



1) LAB and HPB co-existence in DF reactors
2) HPB emerged even after low pH storage

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Conclusions

- Food waste can be stored in a wide range of temperatures in LAF without affecting its biohydrogen potential (BHP)
- Requires no energy for temperature maintenance during storage
- Indigenous Hydrogen Producing Bacteria can withstand low pH environment during storage and re-emerge after
- Enables decentralized storage system and demand-oriented collection [10]
- Has potential to manage seasonality of food waste.

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[10] M. Wehner et al., "Decentralised system for demand-oriented collection of food waste - assessment of biomethane potential, pathogen development and microbial community structure," Bioresour Technol, vol. 376, no. March, p. 128894, 2023, doi: 10.1016/j.biortech.2023.128894.

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Questions?
Thank you!

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