BACTERIAL COMMUNITY CHARACTERIZATION IN VFAs PRODUCTION FROM INDUSTRIAL WASTE

Laura Dorado Torres
Project/Research Group: Biobased Building Blocks and Products & Biobased Resources and Energy
Contact information: l.doradotorres@avans.nl, mwj.raats@avans.nl
Date: 12 June 2025

Introduction

The growing environmental impact of waste and resource depletion highlights the need for sustainable solutions such as **anaerobic digestion (AD)**, a biological process that transforms organic waste into valuable products like **volatile fatty acids (VFAs)** (United States Environmental Protection Agency, 2024). VFAs are key intermediates with wide industrial applications and can be efficiently produced from organic waste through AD (Atasoy *et al.*, 2018; Feng *et al.*, 2022).

This study investigates the anaerobic digestion of wheat processing waste using secondary sludge from wastewater treatment as inoculum. By using **Oxford Nanopore sequencing** (Oxford Nanopore Technologies, n.d.), we analyzed the **bacterial communities** involved in semi-continuous and batch fermentations to understand their role in the metabolic pathways of VFA synthesis. This microbial insight contributes to a better understanding of how **environmental parameters,** such as pH and temperature, influence bacterial biodiversity in VFAs production. (Tampio *et al.*, 2019; Solera del Río & Álvarez Gallego, 2014).

Methodology

To characterize the bacterial communities involved in **VFA production** from **starch-based** industrial waste, the following methodological approach was implemented.

Samples were collected from **semi-continuous fermentation** process operating in bioreactors and **batch fermentation** process operating in bottles. Subsequently, **metagenomic DNA** extraction was performed using specialized commercial kits, followed by rigorous assessments of DNA quality, quantity, and fragment length to ensure suitability for downstream analysis.

For **taxonomic profiling**, a **DNA library** was constructed and sequencing was carried out using **Oxford Nanopore Technologies** devices. The resulting sequencing data were analyzed using the **Strainoscope pipeline**, allowing for high-resolution identification and classification of bacterial communities present in the fermentation samples.

Discussion of the results

Regarding the experiments carried out in **bioreactors**, a clear difference in bacterial biodiversity can be observed between bioreactor 1 and 2. In this case, the **pH** of bioreactor 1 is uncontrolled, while the pH of bioreactor 2 is 5.5.

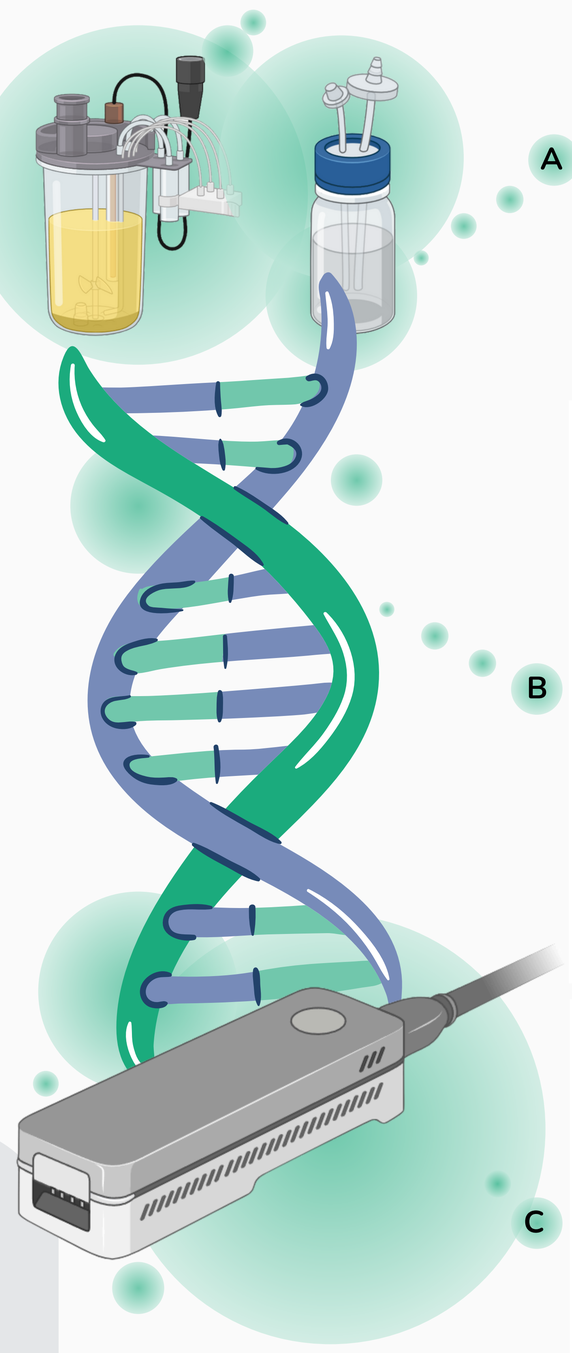
Regarding the **bottle experiments**, the key parameters assessed were pH and temperature. Bottle designs 7 (pH 6.0) and 15 (pH 6.4) demonstrated reduced bacterial diversity, likely due to the elevated pH levels. In contrast, bottle designs 9 and 10, which were duplicated and operated at pH 5.0 and 35°C, showed more favorable biodiversity results.

Bioreactors		
	pH	Temp (°C)
1	Uncontrolled	35
2	5.5	35
Bottles		
	pH	Temp (°C)
7	6	40
9	5,5	35
10	5,5	35
15	6,4	35

Table 1. pH and temperature parameters used in the different experimental designs.

Furthermore, in all experimental designs, ***Lactobacillus*** and ***Acetobacter*** are the most dominant and persistent genera throughout the fermentation process.

Notably, there is a positive correlation between the diversity of bacterial species identified and the **production of VFAs**.



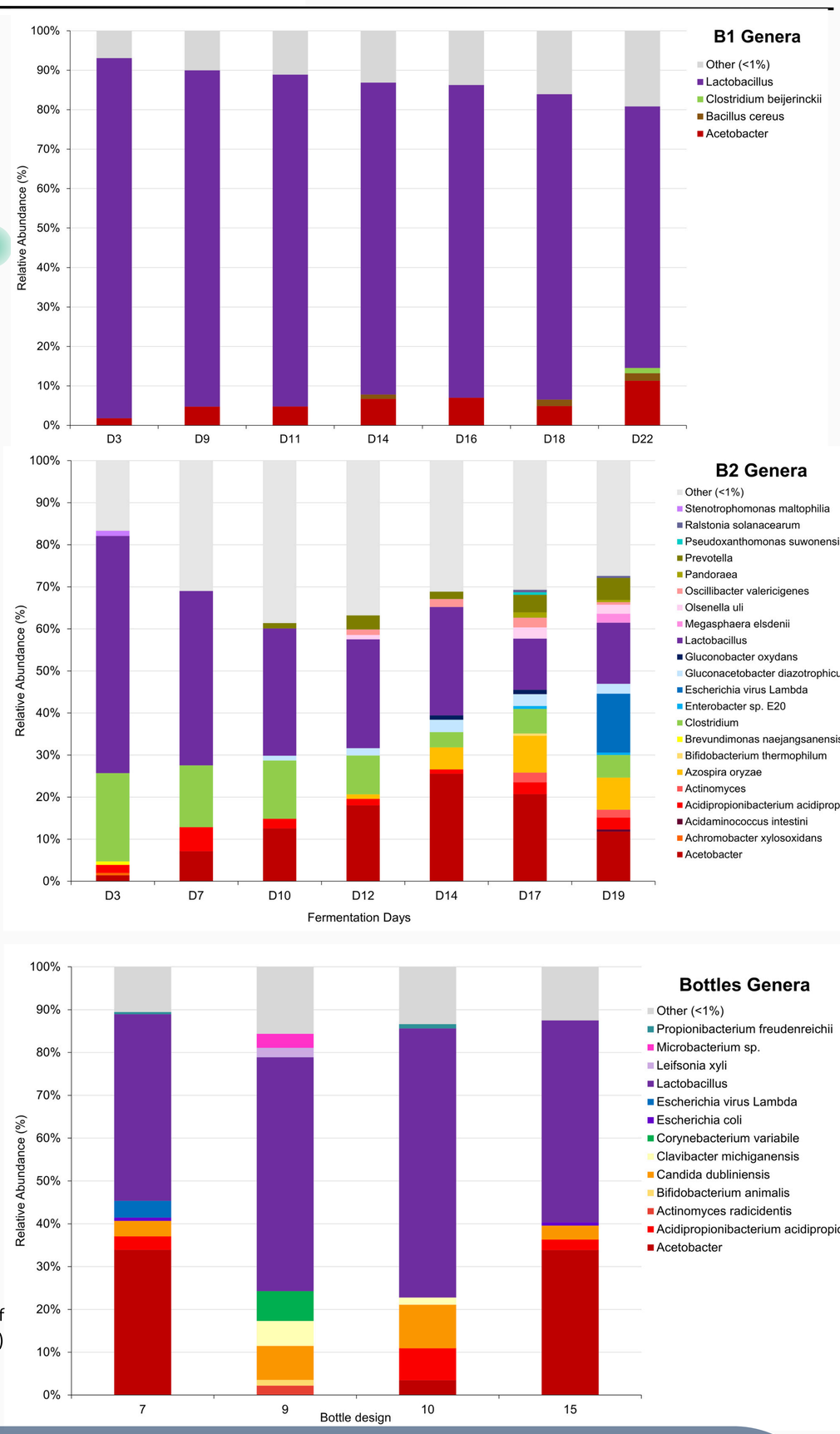


Figure 1. Results of the characterization of bacterial communities. A) Bioreactor 1 B) Bioreactor 2 C) Experimental designs in bottles

Conclusions

This project demonstrates a clear influence of parameters such as pH, temperature and inoculum concentration on the bacterial profile present in the production of VFAs from wheat processing waste with secondary sludge inoculum from water treatment.

Regarding increased bacterial biodiversity, pH 5.5 and temperature 35°C are the most favorable parameters for the anaerobic digestion of wheat processing waste, higher or uncontrolled pH being a limiting factor.

References

- United States Environmental Protection Agency (2024). How does anaerobic digestion work?
- Atasoy *et al.* (2018). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges.
- Feng *et al.* (2022). Volatile fatty acids production from waste streams by anaerobic digestion: A critical review of the roles and application of enzymes.
- Oxford Nanopore Technologies (n.d.). How nanopore sequencing works.
- Tampio *et al.* (2019). Volatile fatty acids (VFAs) and methane from food waste and cow slurry: Comparison of biogas and VFA fermentation processes.
- Solera & Álvarez Gallego (2014). Aspectos biológicos de la digestión anaeróbica II.

Acknowledgements

I would like to express my sincere gratitude to my supervisors Mirthe Raats, Tim Verschuren, Sára Finta, Bazante Sanders and Mithyzi Andrade Leal.

This Project is financially supported by the European Just Transition Fund via project **FermiChem** (JTFZ-00022) and by imPHAct! Project via TKI ChemistryNL(CHEMIE.PGT.2023.036).