Bacterial carbon monoxide (CO) cycling via the CODH/ACS complex in compost: insights from metagenomic and gene expression analyses

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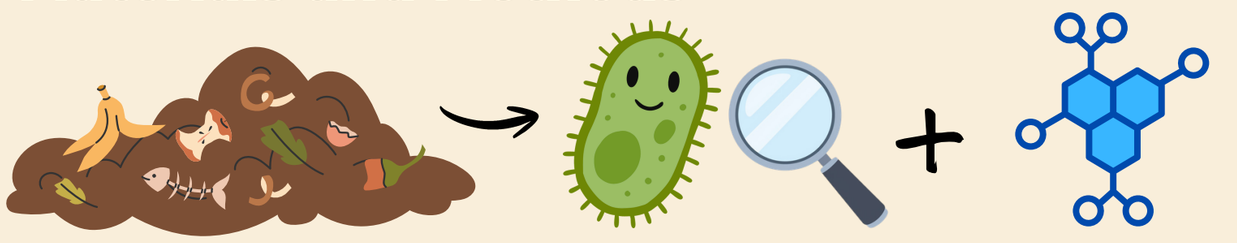
Introduction

Bacterial production of carbon monoxide (CO) during biowaste composting may represent a cost-effective and environmentally friendly strategy for CO generation in waste-based biorefineries.
CO is produced by bacteria via the bidirectional enzyme CO dehydrogenase (CODH). In aerobic microorganisms, CODH functions in association with acetyl-CoA synthase (CODH/ACS, encoded by the *acsAB* genes). Within the Wood–Ljungdahl pathway, CO is generated from CO_2 by *acsA* and subsequently converted to acetyl-CoA by *acsB*. Despite the recognized role of this pathway in microbial metabolism, its involvement in composting processes has not yet been directly investigated.

Therefore, this study aimed to characterize the microbial community and examine the expression of the *acsA* and *acsB* genes during biowaste composting, providing the first direct evidence of CODH/ACS activity in this process.

Materials and Methods



Biowaste composting in the laboratory scale | **Microbial community profiling in compost samples** | ***acsAB* genes expression analysis in compost samples**

Results

- At D0, early-stage communities were characterized by higher relative abundances of taxa such as *Pantoea agglomerans*, *Escherichia coli*, and *Citrobacter freundii* (Fig. 1).
- During the process (D7–D14), an increase in *Bacillus* spp. and *Saccharomonospora* spp. was observed, including species previously reported to be capable of CO production, such as *Bacillus subtilis* and *Bacillus licheniformis* (Fig. 1).

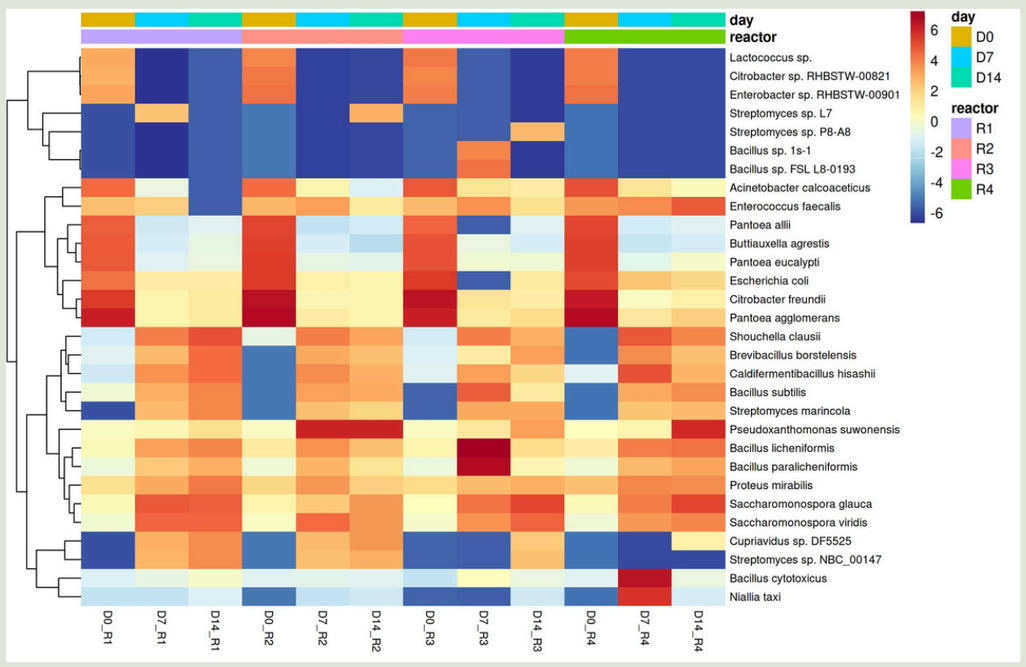


Figure 1. Relative abundances of the top 30 species across composting time points (D0, D7, D14)

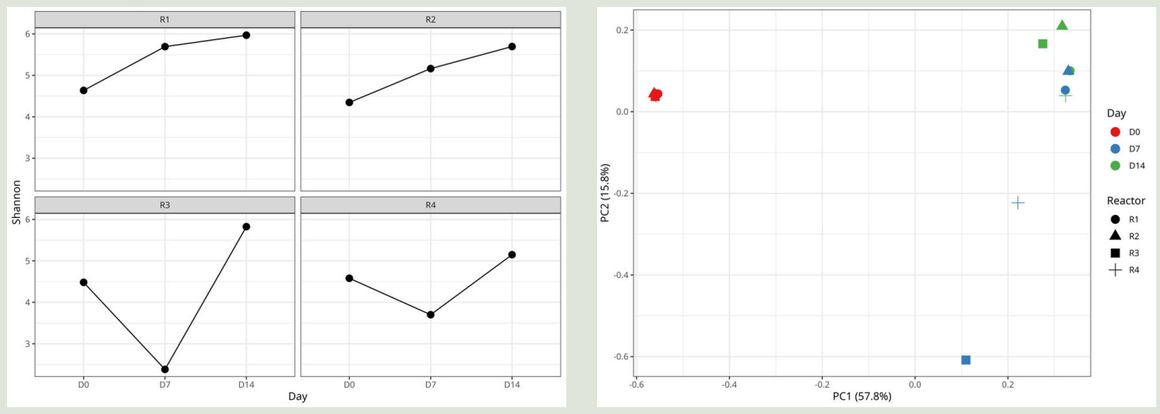


Figure 2. Shannon diversity (Bracken, species) (left) and PCoA (Bray–Curtis) of species composition (right) in compost samples

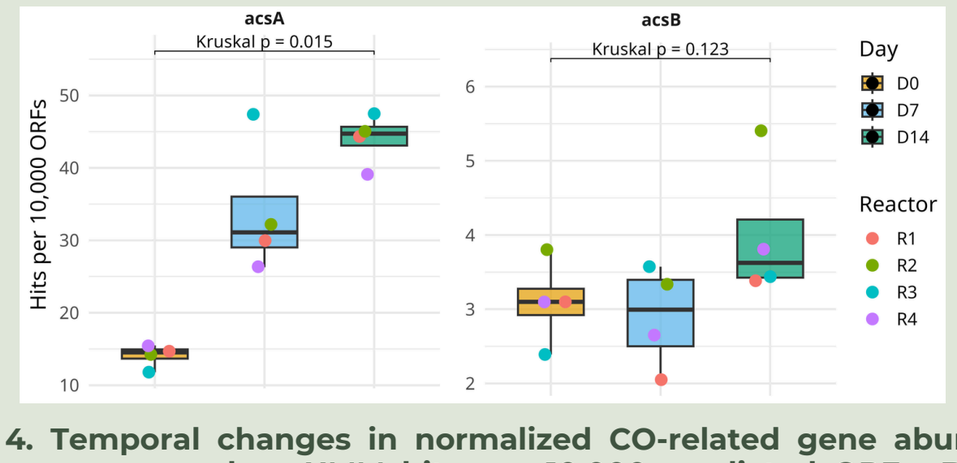


Figure 4. Temporal changes in normalized CO-related gene abundance. Values are expressed as HMM hits per 10,000 predicted ORFs. Boxplots show reactor distributions, points represent individual reactors, and Kruskal–Wallis global p-values are given for each gene.

- The Shannon index indicated temporal changes in microbial diversity and evenness (Fig. 2). Diversity increased over time in R1 and R2, while R3 and R4 showed a temporary decrease on day 7 followed by recovery on day 14.
- PCoA based on Bray–Curtis dissimilarity (Fig. 2) revealed clear separation between day 0 samples and later stages along PC1 (57.8% variance explained). Samples from days 7 and 14 clustered more closely, indicating increasing similarity of microbial communities over time, despite transient differences observed in R3 and R4 on day 7.
- To evaluate shifts in metabolic potential, CO-related genes were quantified as hits per 10,000 predicted ORFs (Fig. 4). Temporal analysis showed significant enrichment of *acsA*, increasing from ~10–20 hits at day 0 to ~40 hits by day 14 (Kruskal–Wallis, p = 0.015). In contrast, *acsB* abundance did not change significantly over time (p = 0.123 and 0.304).

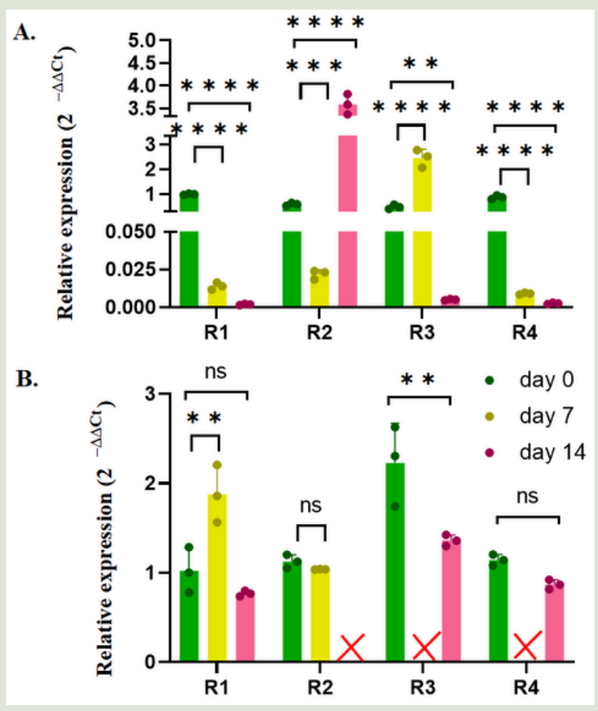


Figure 5. Temporal changes in relative expression of *acsA* (A) and *acsB* (B) genes during composting; p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), and p < 0.0001 (****)

- *acsA* expression generally decreased during incubation, with significant reductions observed in R1 and R4 after day 7 (Fig. 5). In contrast, R3 showed a transient increase at day 7, while R2 reached the highest acsA expression at day 14.
- By day 14, *acsB* expression decreased in all analyzed reactors, although a statistically significant reduction was observed only in R3.

The results confirm that the CODH/ACS pathway is active during biowaste composting, indicating the ability of microbial communities to perform CO-related metabolism. Temporal changes in *acsA* and *acsB* expression revealed dynamic regulation of this pathway, while shifts in microbial community composition suggested enrichment of taxa potentially involved in CO transformation. Differences between reactors indicate that CO metabolism is influenced by local composting conditions and community structure.

Let's stay in touch!

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