



# Insights into prokaryotic metataxonomics and predictive metabolic function in field-scale anaerobic digesters treating various organic wastes

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## ABSTRACT

The operational temperature and nature of the substrates are two critical parameters in shaping the functional microbiota and determining the biogas productivity of field-scale anaerobic digesters. Elucidating microbial ecological configurations and dynamics as a function of parametric (temperature, pH, substrate variability etc.) variations is pivotal for effective process management, improving the process stability and digestion efficiency of large field-scale anaerobic digesters. Seventeen field-scale digesters treating food waste leachate, municipal wastewater sludge (MWS), and manure at 10 different sites across the Republic of Korea were studied to assess how substrate variations, including co-digestion and seasonal variation, affect biogas productivity and the compositional dynamics of the digester microbiota. The 16S rRNA gene metataxonomics and predictive metabolic pathway profiling revealed significant differences in the phylogenetic composition of the microbiota between the digester types depending on the presence of MWS in the substrate ( $p < 0.005$ ). A clear compositional divergence was observed between the MWS and non-MWS substrate groups, with a high prevalence of Firmicutes and versatile organic matter-degrading genera (*Fastidiosipila*, *Acholeplasma*, etc.) in the non-MWS digesters, resulting in a significantly high biogas yield (53.4 Nm<sup>3</sup>/t). Predictive metabolic pathway profiling demonstrated the importance of acetoclastic methanogenesis in all anaerobic digestion groups subjected to the study. The results of this study suggest that metataxonomics and microbiota dynamics are good indicators of the process stability and efficiency of field-scale digesters across the parametric variations of field anaerobic digester systems.

**Abbreviations:** μL, microliter; 16S rRNA, 16S ribosomal ribonucleic acid; AD, Anaerobic digestion; ADS, anaerobically digested sludge; ASV, Amplicon sequence variant; COD, Chemical oxygen demand; DADA2, Divisive Amplicon Denoising Algorithm; DJ, Daejeon; FWL, Food waste leachate; g/L, Grams per liter; GTDB, Genome Taxonomy Database; HC, Hongcheon; JR, Jungnang; KN-SC, Sokcho; L, Liter; LCFA, Long chain fatty acid; MAFFT, Multiple Alignment using the Fast Fourier Transform; mg/L, milligrams per liter; MWS, Municipal wastewater sludge; NCBI, National center for biotechnology information; ng/μL, nanogram per microliter; NJ, Nanji; Nm<sup>3</sup>/yr, Normal cubic meter per year; PCoA, Principal coordinate analysis; PERMANOVA, Permutational analysis of variance; pmol, picomolar; QIIME, Quantitative Insights into Microbial Ecology; SC, Sacheon; SCOD, Soluble chemical oxygen demand; SDS, Sudokwon Landfill Site; SAO, Syntrophic acetate oxidizer; SFAO, Syntrophic fatty acid oxidizer; t/d, tons per day; TCOD, Total chemical oxygen demand; TN, Total nitrogen; TS, total solids; VS, volatile solids; WJ, Wonju; YC, Yeongcheon; YS, Yangsan.

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