

Identifying rate limiting enzyme steps

Using RNA sequencing to identify rate limiting enzymatic reaction steps and reconstruct metabolic networks in anaerobic digestion (AD)

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Background

Catabolism is the biological breakdown of complex molecules to smaller ones to release energy. Hydrolases, Lyases and Transferases are all groups of **enzymes** which perform this essential function in AD systems and are considered the **rate limiting** aspect. This study monitored enzyme **gene expression** over time in a microbial community after feeding by sequencing the short lived biological signaling molecules called **mRNA**.

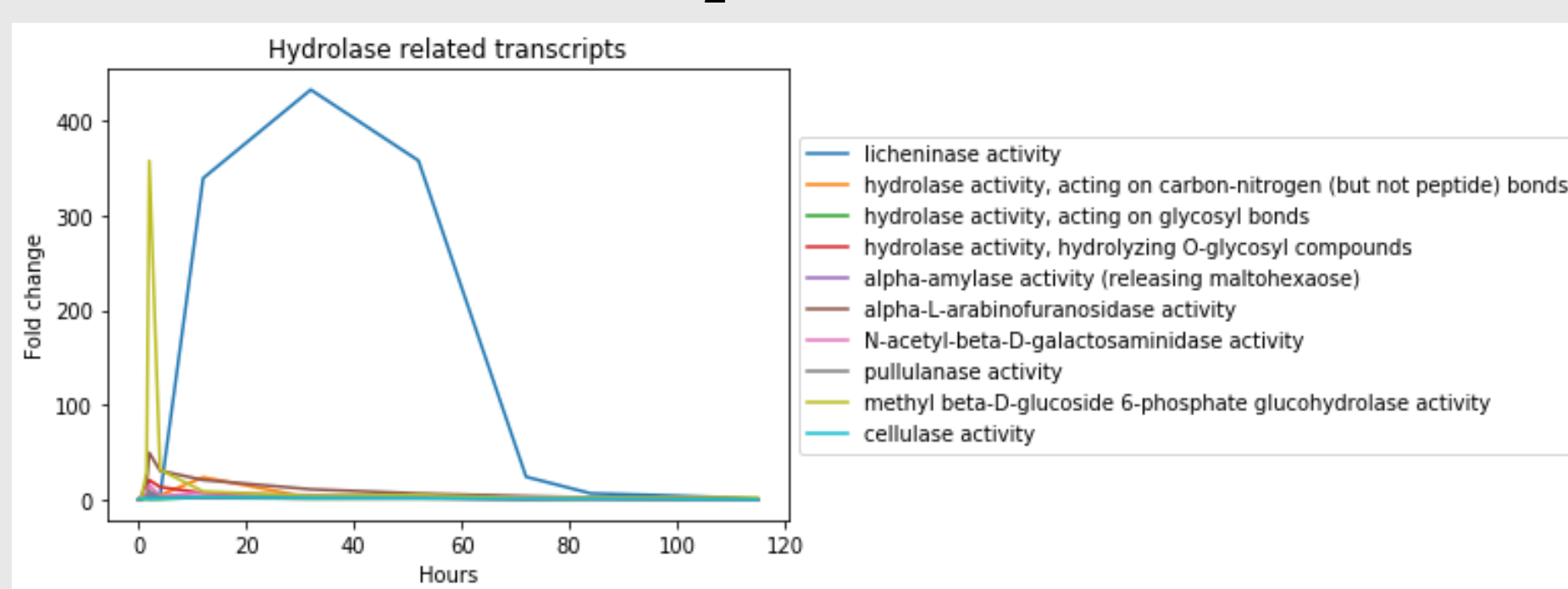
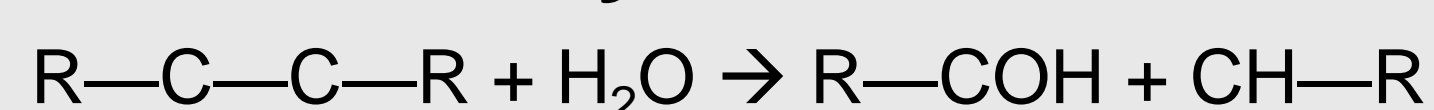
Methods

1. Reactors were fed pot ale, a whisky distillation waste, and sampled for mRNA over the next 120 hours
2. 351 million mRNA fragments were Illumina sequenced and assembled into 471,000 transcripts using York's **Viking** supercomputer
3. Transcripts were quantified then phylogenetically and functionally annotated before being grouped into enzyme classes

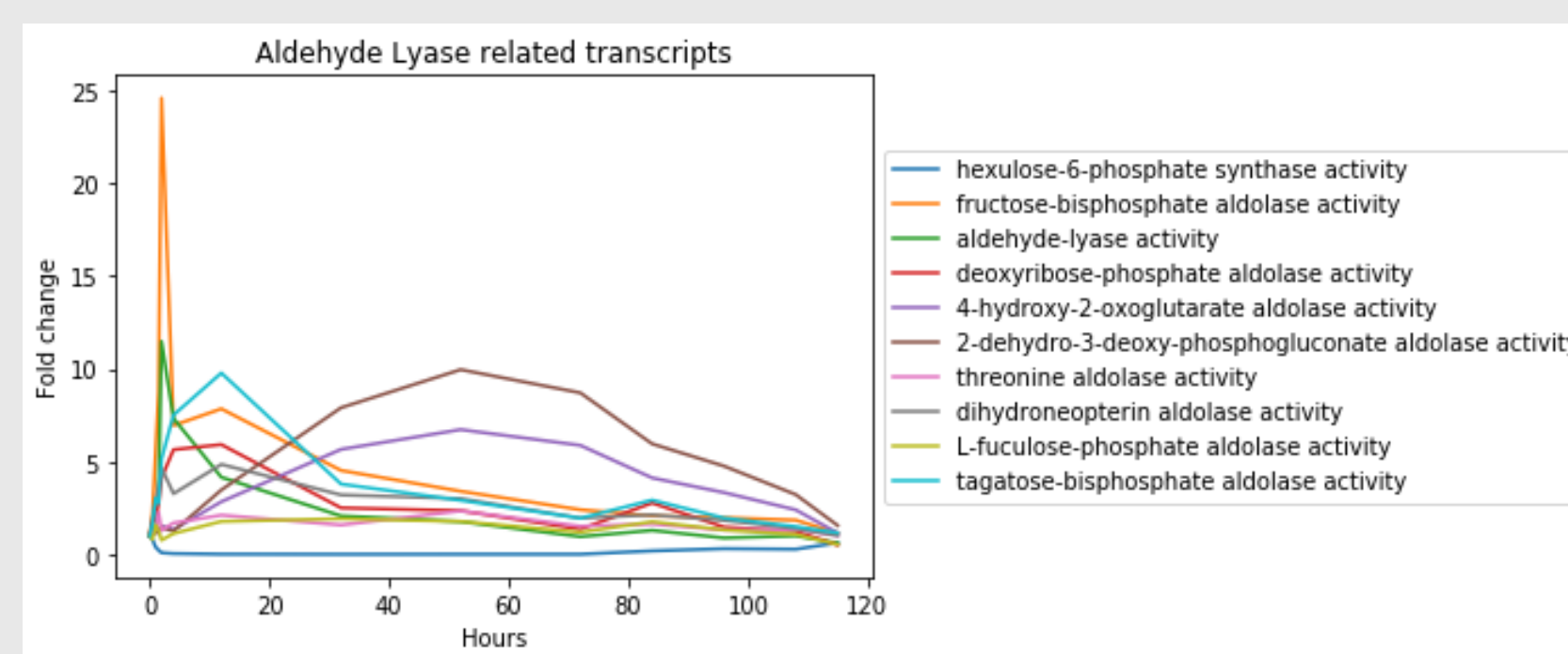
Results

Catabolism

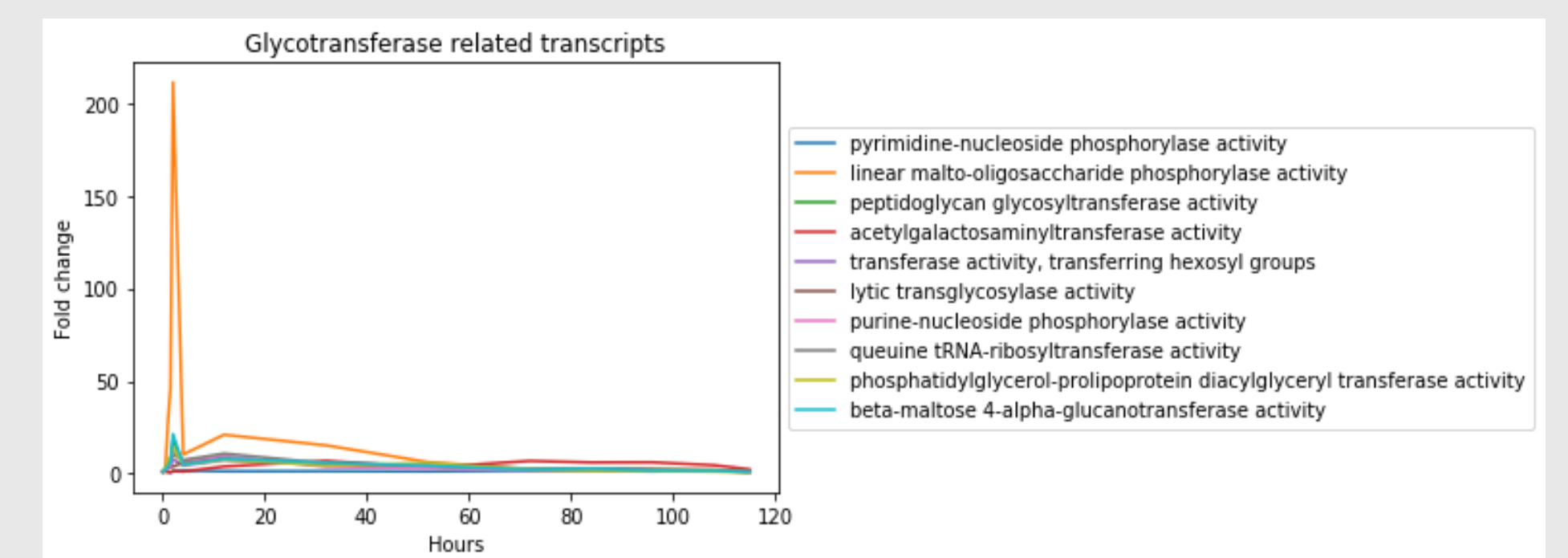
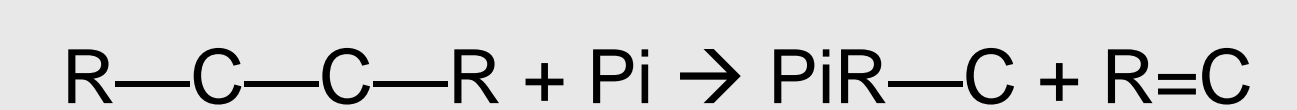
Hydrolases



Lyases

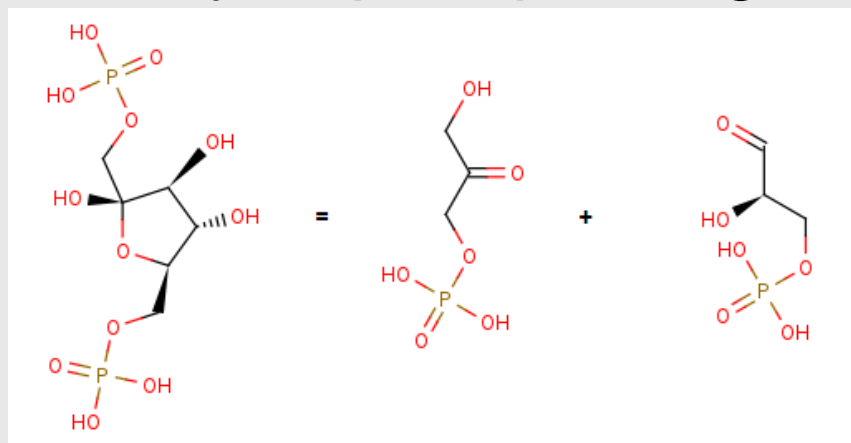


Transferases

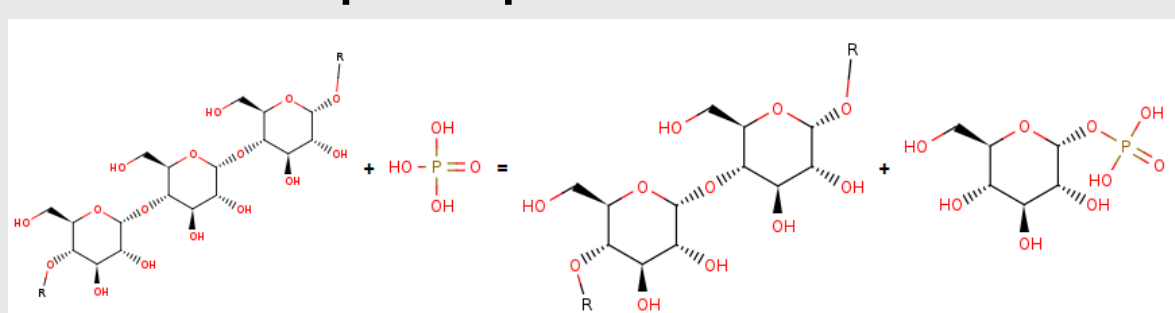


Fast enzyme reactions <4 hrs

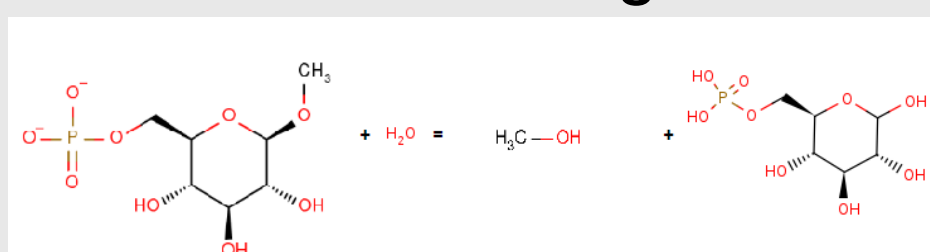
Hydrolase - Methyl 6-phosphate glucosylase



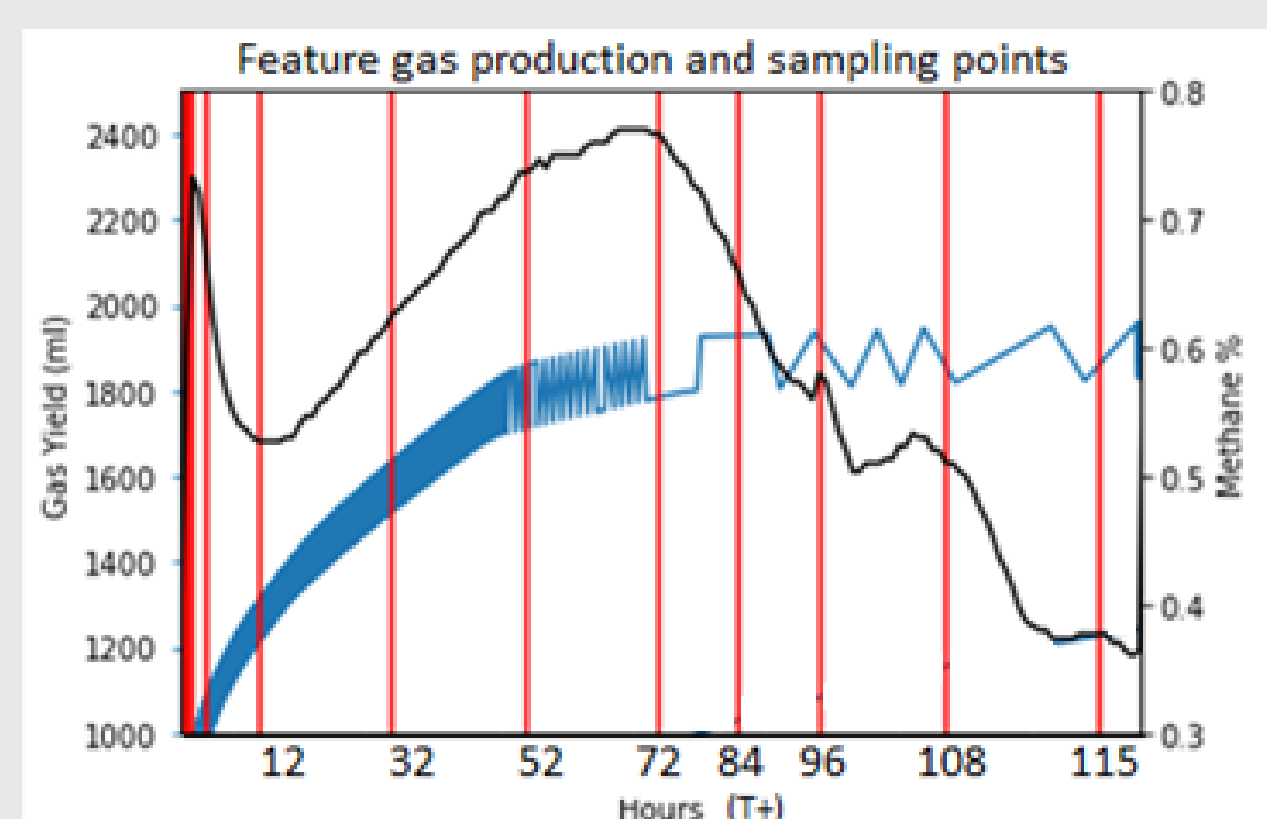
Lyase - Fructose-bisphosphate aldolase



Transferase - Linear malto-oligosaccharide phosphorylase



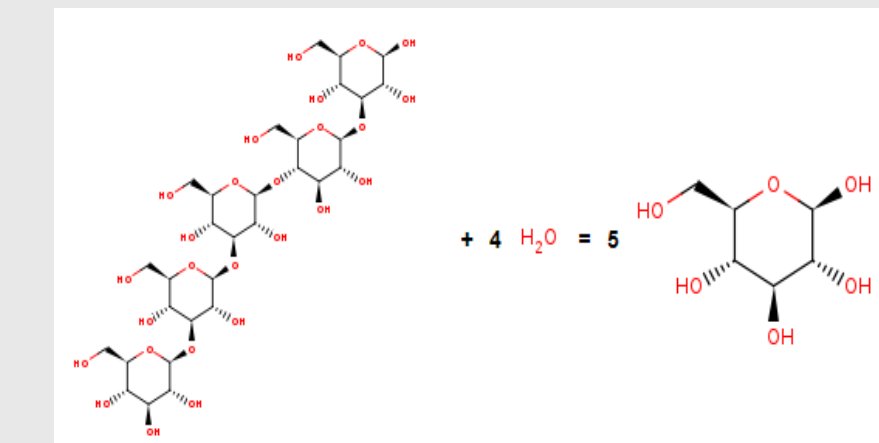
Gas Production



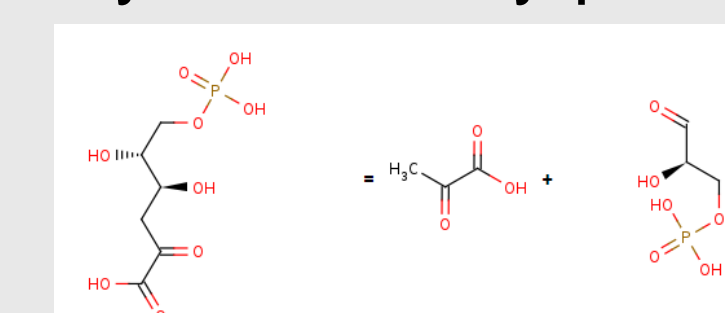
Immediately following feeding (0 hours) a sharp rise in headspace methane concentration is observed (black line) followed by second more gradual increase. Total gas production (blue line) indicates rapid gas production activity within the first 52 hours. Points over this biphasic gas production were selected for further transcriptional analysis (vertical red lines).

Slow enzyme reactions >40 hrs

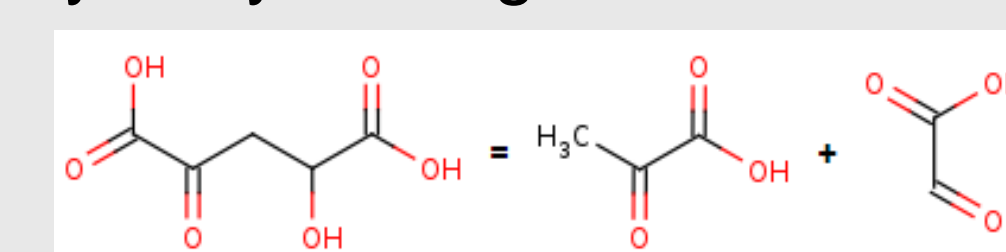
Hydrolase - Licheninase



Lyase - 2-dehydro-3-deoxy-phosphogluconate aldolase



Lyase - 4-Hydroxy-2-oxoglutarate aldolase



Conclusions and Discussion

- **RNA sequencing** provides unprecedented insight into the active processes and populations essential for AD by revealing exactly what processes are occurring at any given time rather than what may be occurring based on DNA sequence
- Expression analysis revealed two main catabolic processes occurring; the **rapid** <4 hr degradation of residual sugars followed by the slow >71 hr **rate limiting** degradation of beta-glucans
- Metabolic pathways associated with expressed enzymes were reconstructed to identify potentially valuable **intermediary products** which could be extracted as part of a **bio-refinery**
- This analysis pipeline could be extended to any other feedstocks to identify rate limiting enzymatic steps for any system



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