



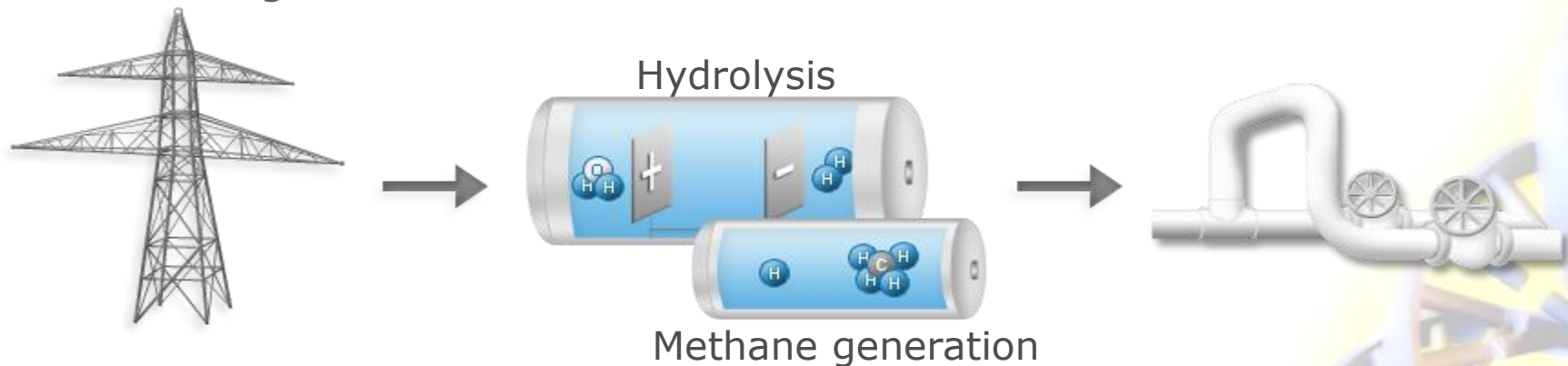
Metagenomic rearrangement of the biogas producing community during hydrogen assisted in-situ biogas upgrading

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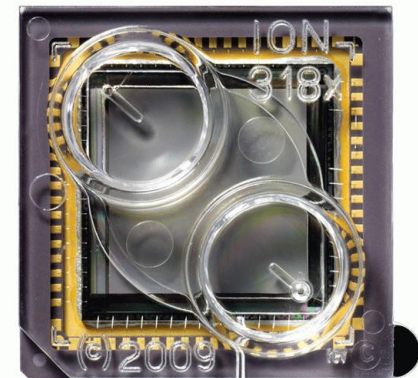
The P2G theory in practice

- Excess power is generated during periods of low power consumption and fluctuating production
- This electricity is used for hydrolysis of water
- H_2 is introduced into the biogas reactor directly, into a separate reactor or into the **post fermentor** tank
- Upgraded biomethane (purity: $\sim 90\text{-}98\%$) is produced
- Biomethane can be injected into the natural gas grid for transport or storage



Experimental set-up

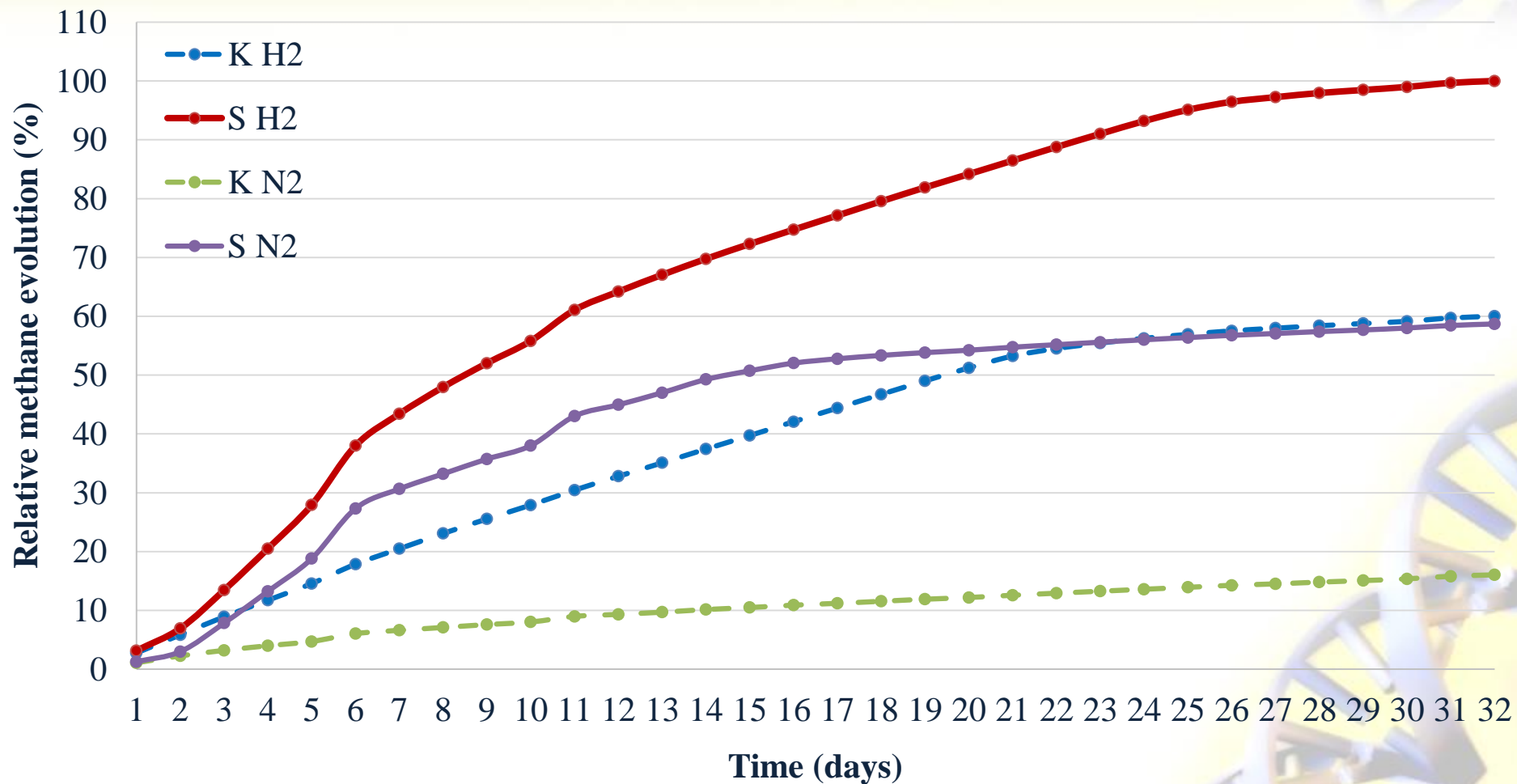
- The role of H_2 in anaerobic fermentation has been in our research interest
- **Fed-batch** fermentations to examine the effects of **H_2 feeding**
- Monitoring the key process parameters and the microbial community
- **T-RFLP** fingerprinting and **IonTorrent™** next generation sequencing



Samples


Sample name	organic substrate	H ₂ addition	Duration (weeks)
Inoculum			0
KH ₂		✓	4
SH ₂	✓	✓	4
KN ₂			4
SN ₂	✓		4
KH ₂ w12		✓	12
KN ₂ w12			12

Methane production

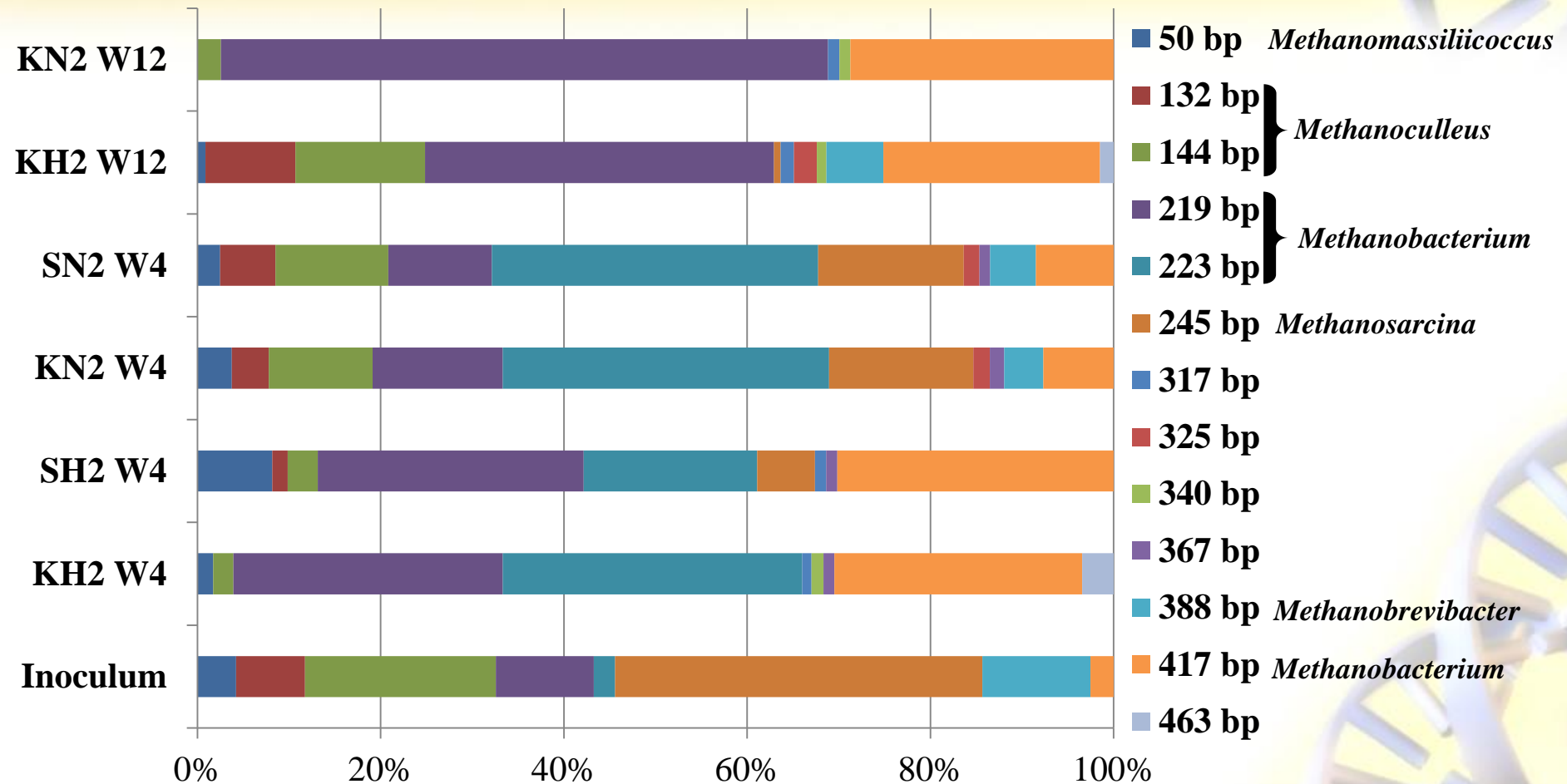


Molecular methods




- IonTorrent TM PGM
 - whole-genome shotgun sequencing mode (both taxonomical and functional data)
 - Ion 318 chip (246.000 – 367.000 reads avg. length: 215 ± 86 bp)
 - annotation on MG-RAST using M5nr database (e-value cut-off: 10^{-5} , min. identity cut-off: 70%, min alignment length cut-off: 25 bp)
 - T-RFLP, a semi-quantitative fingerprinting for the methanogenic archaea
 - target gene: *mcrA* fragment (~ 500 bp)
 - enzymes: BstNI and MboI
 - run on ABI Prism 310 in GeneScan mode
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T-RFLP results - Archaea

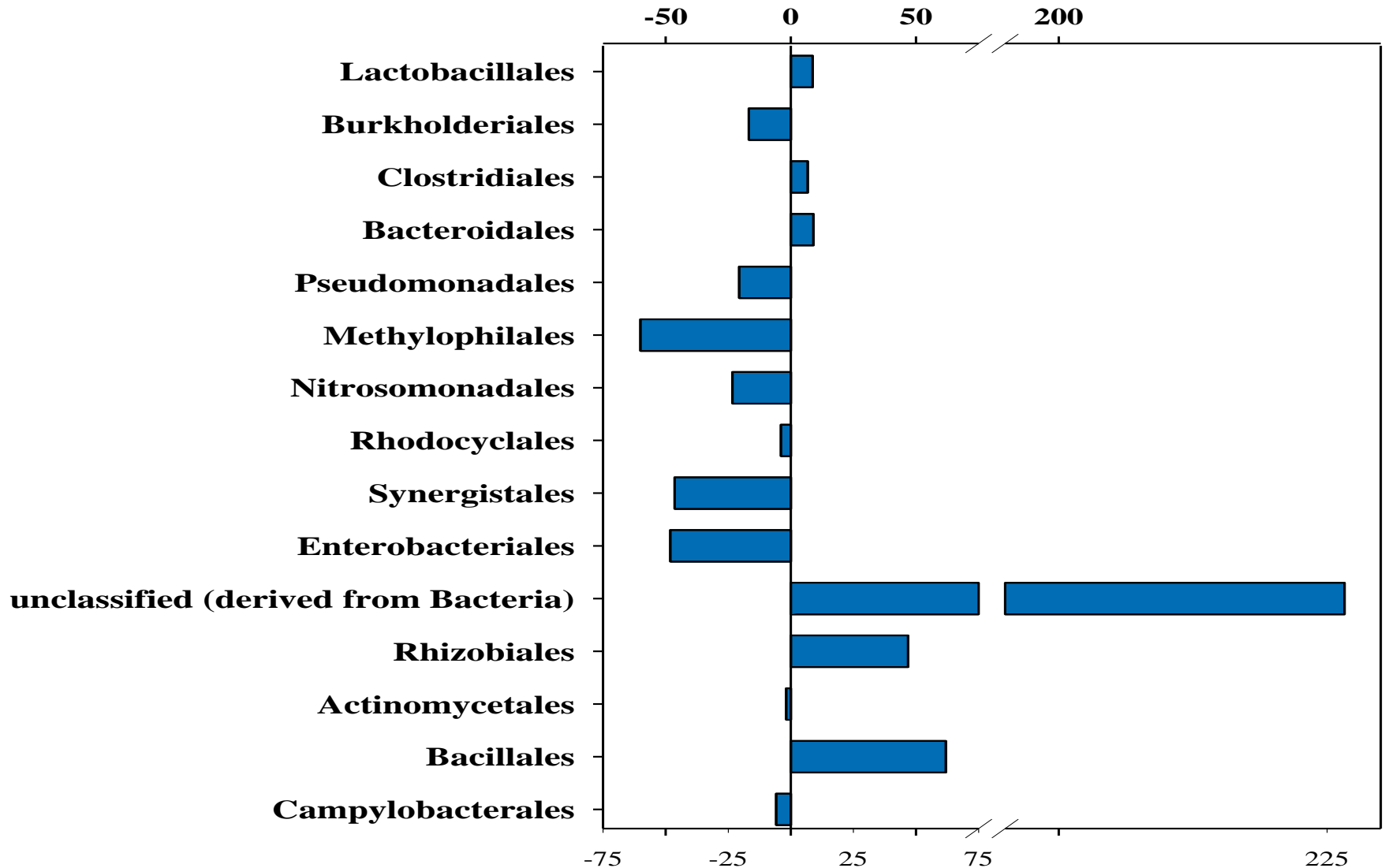


IonTorrent results

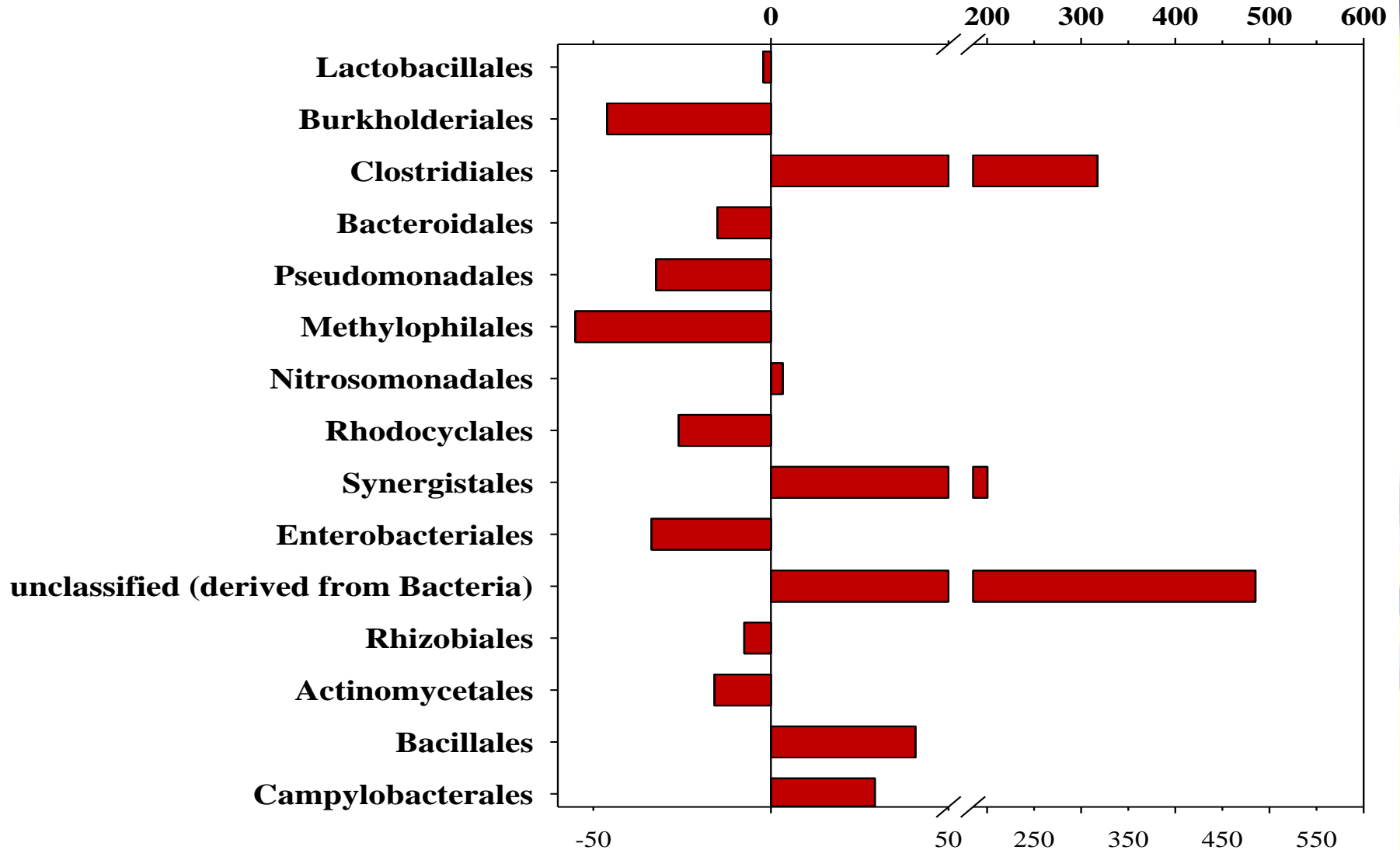


- Representation of the differences in **percent** (-% or +%)
compared to the **relevant control** fermentor
 - In the case of archaea:
 - **~~Strikethrough~~** groups are missing in the control fermentor
 - **Underlined** groups are missing in the H₂ fed sample
 - **Dashed** groups are showing negligible abundance shift
 - Taxonomic levels:
 - Eubacteria: **Order**
 - Archaea: **Genus**
 - More detailed data will be available soon (Ács et. al. 2016, submitted)
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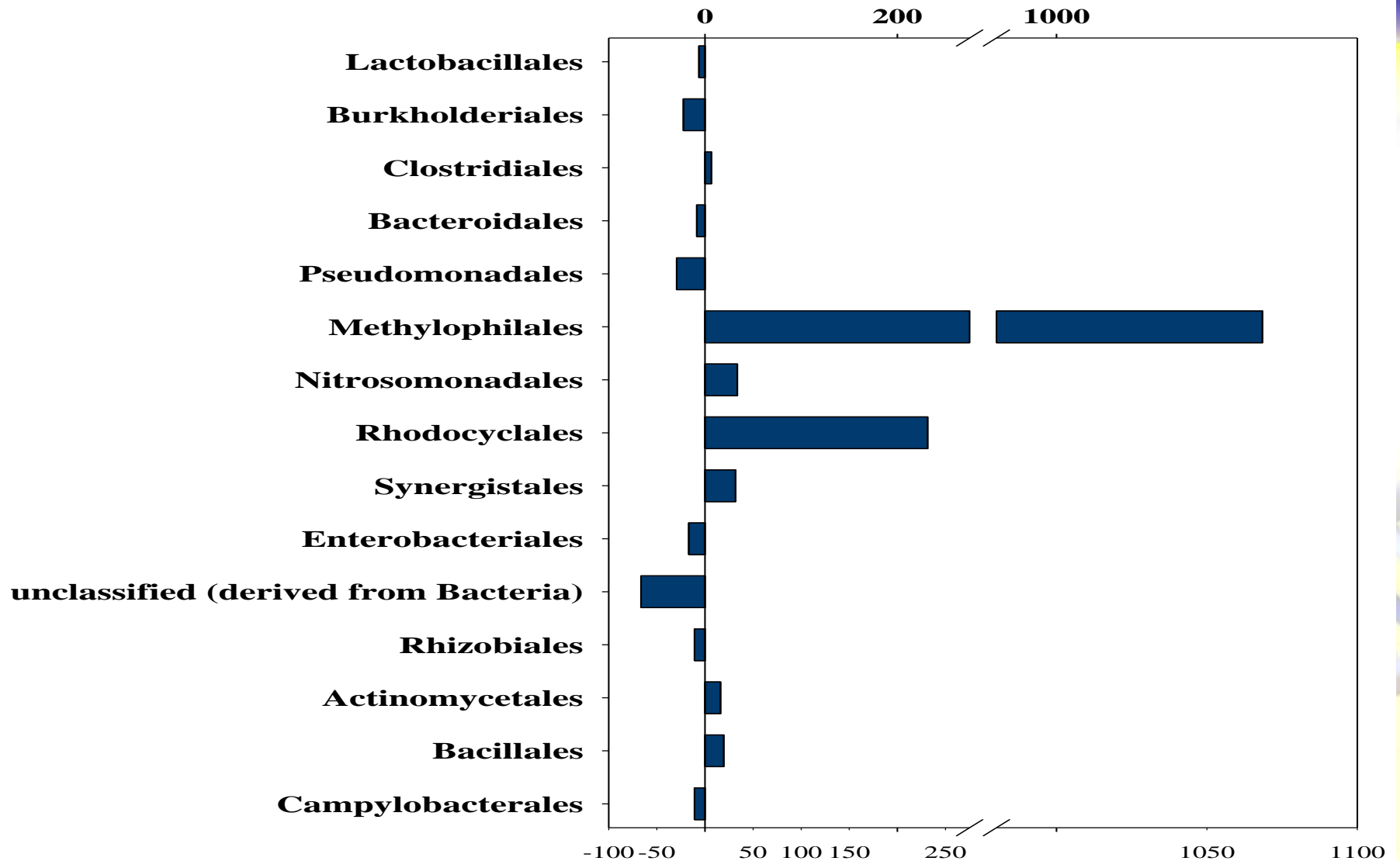
Eubacteria: KH_2 vs. KN_2



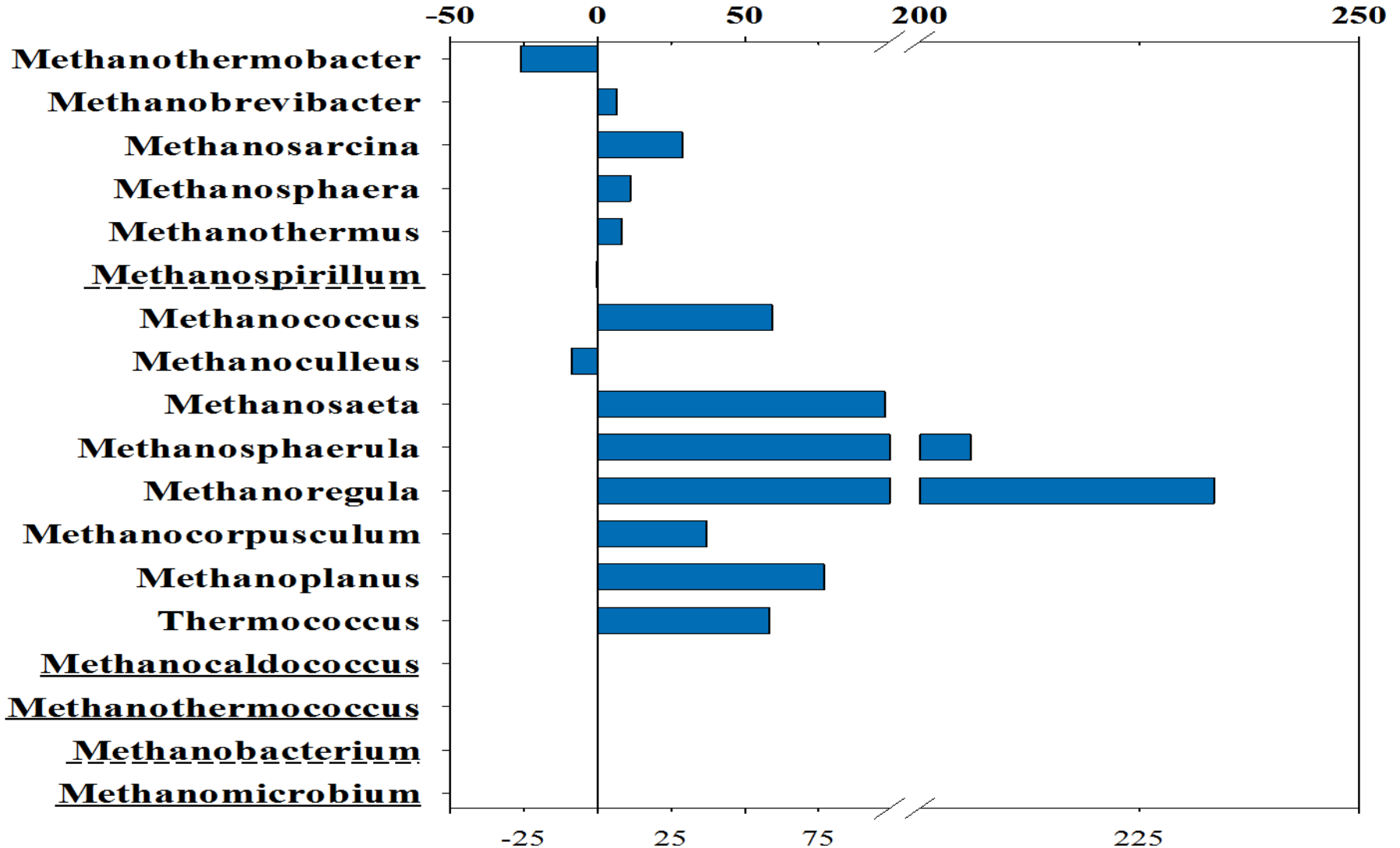
Eubacteria: SH₂ vs. SN₂



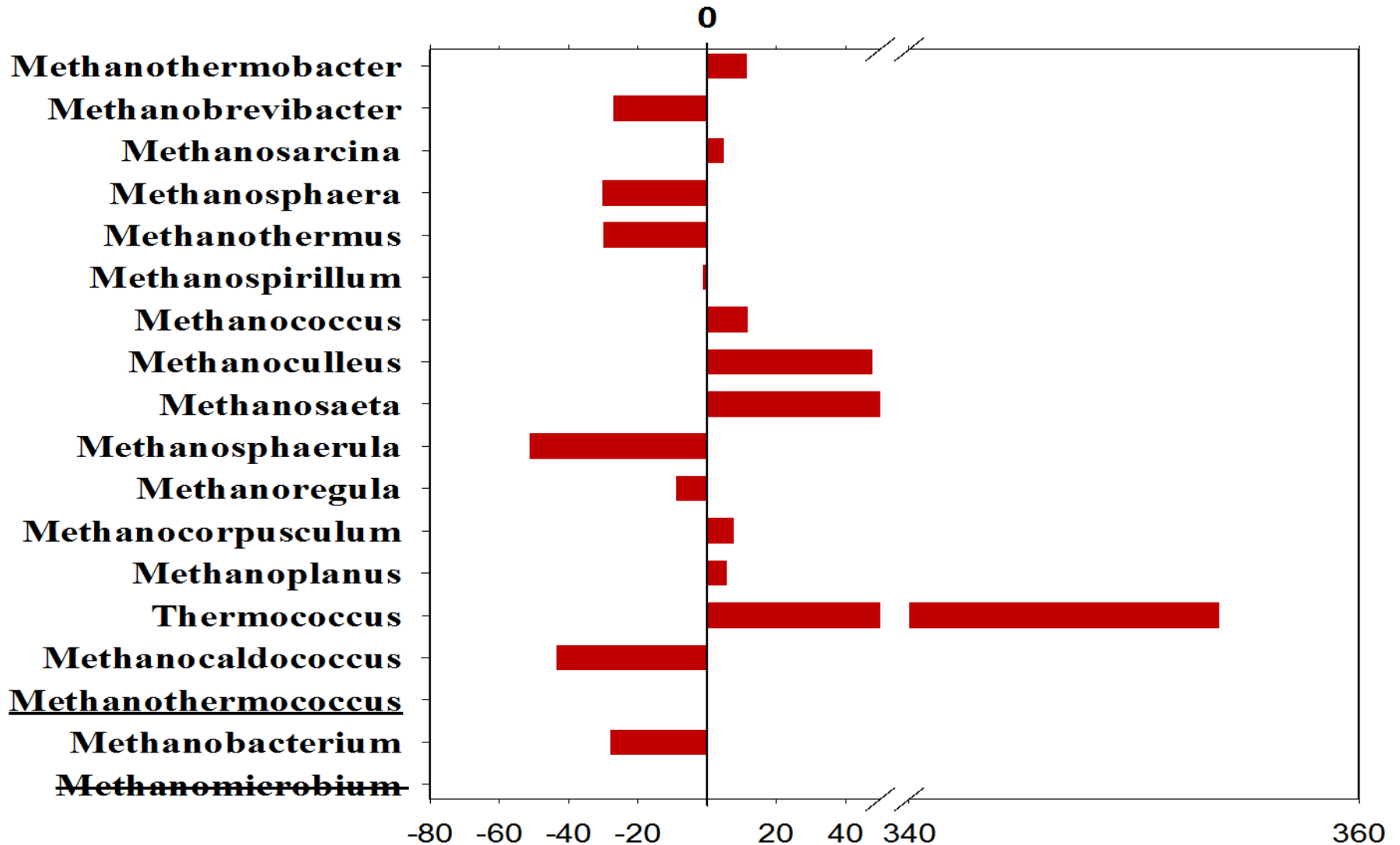
Eubacteria: KH_2w12 vs. KN_2w12



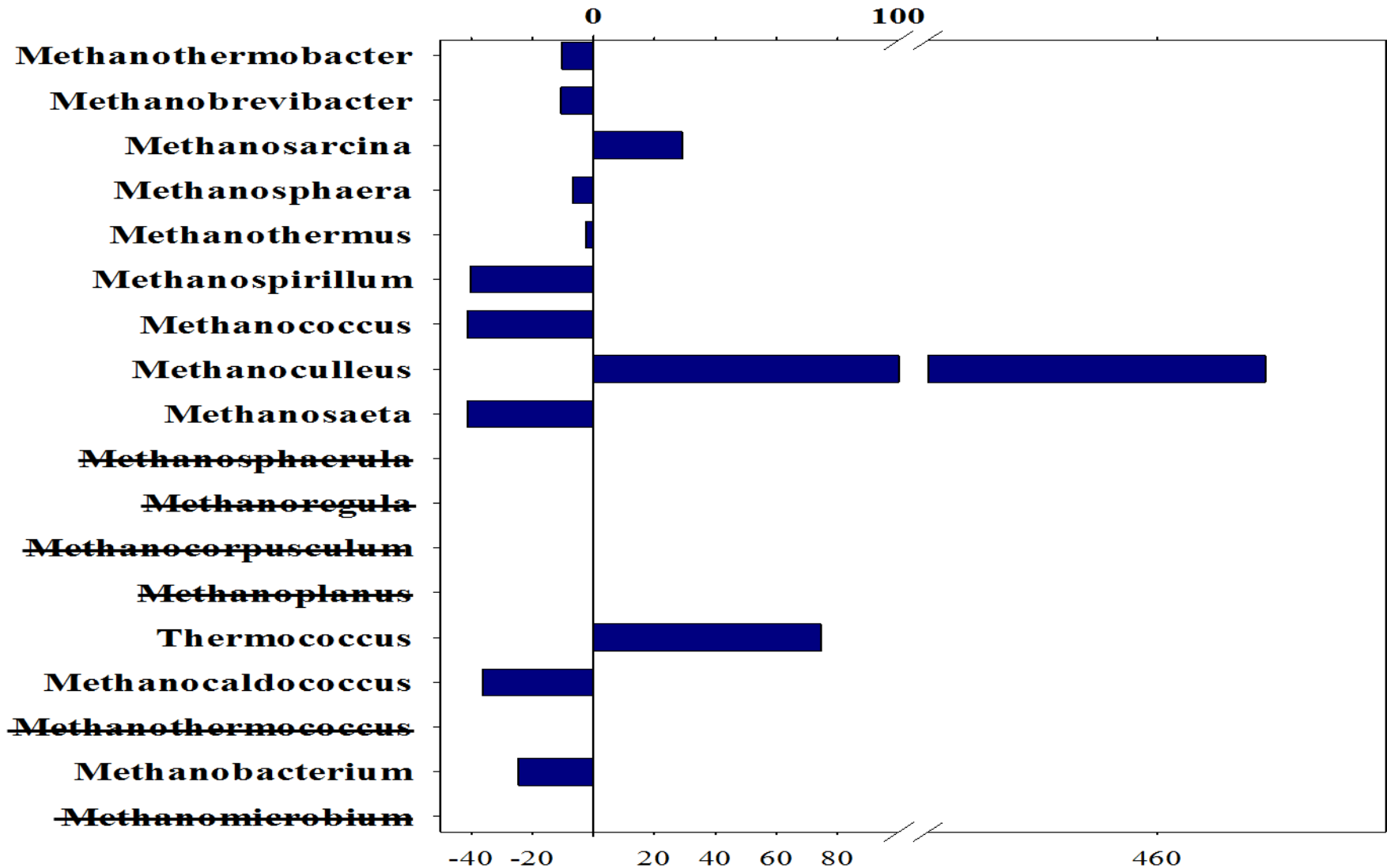
Archaea: KH_2 vs. KN_2



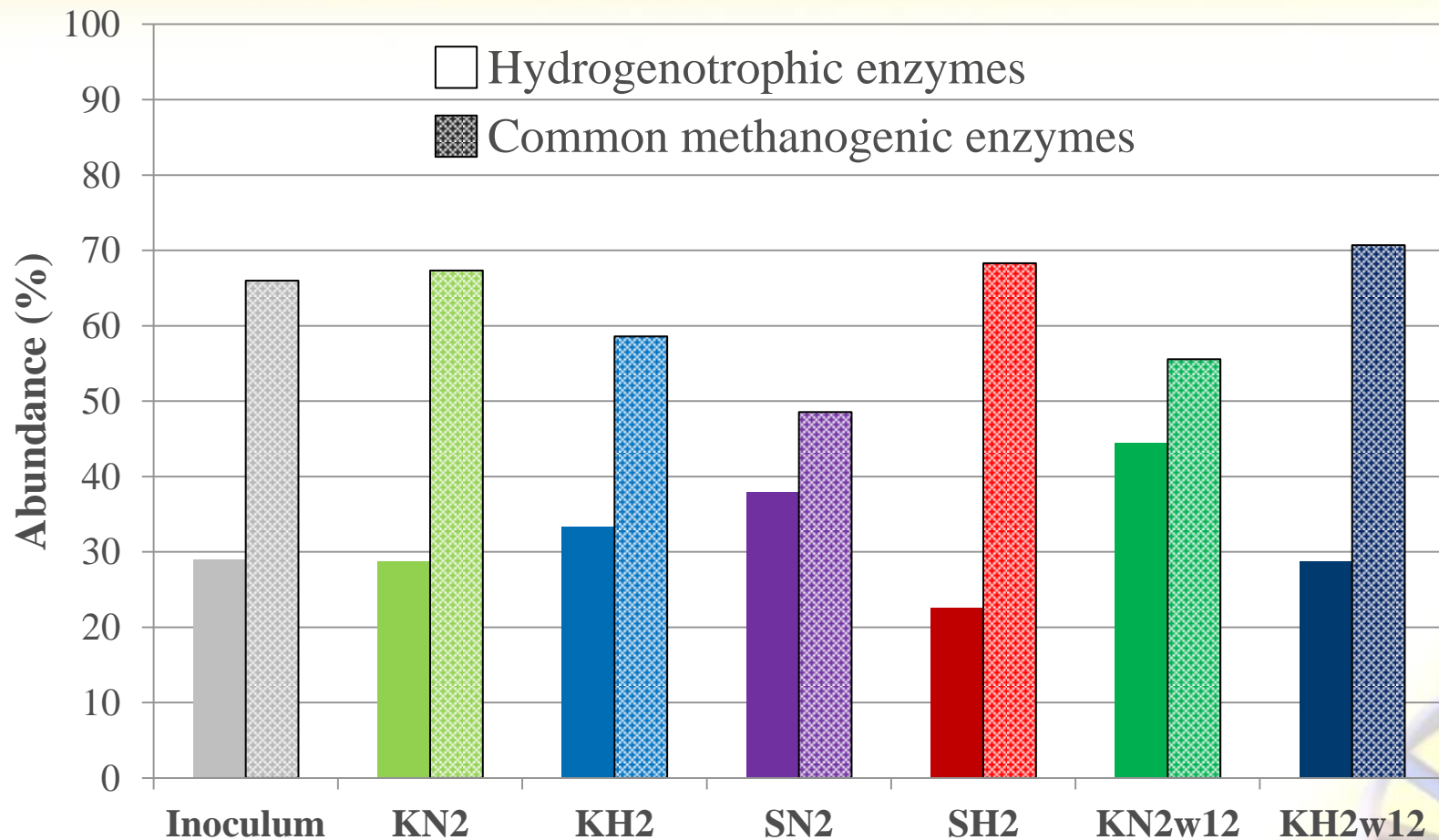
Archaea: SH₂ vs. SN₂



Archaea: $\text{KH}_2\text{w12}$ vs. $\text{KN}_2\text{w12}$



Functional results





Summary

- Biogas upgrading with H_2 (and dissolved CO_2) was accomplished
- Differences at various taxonomic levels in the biogas producing community was demonstrated via IonTorrent PGM and T-RFLP methods
- Differences in functional gene abundances
- The novel P2G scheme may be incorporated into the existing biogas technologies



Thank you for your kind attention!

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More details about the fed-batch fermentation:

Márk Szuhaj et al. - *A novel biotechnological route for the
power to gas concept*

Wednesday (24th August) 10²⁰ - 10⁴⁰