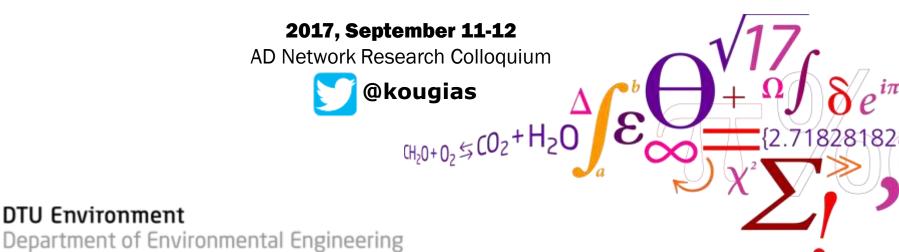


Biomethanisation - opportunities for anaerobic digestion

Panagiotis Kougias



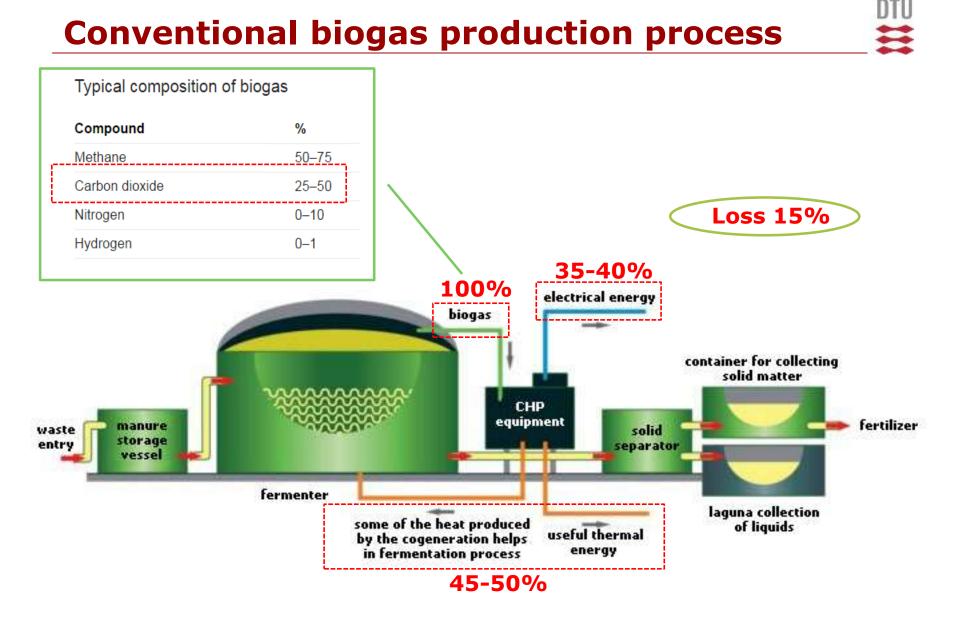


Figure obtained by http://www.bteam-energy.ro/en/information/biogas (last access 07/09/2017)

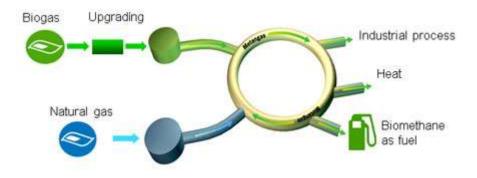
Biogas upgrading



"The process in which the final output gas consists of higher methane concentration compared to raw biogas, mainly due to removal or transformation of carbon dioxide"

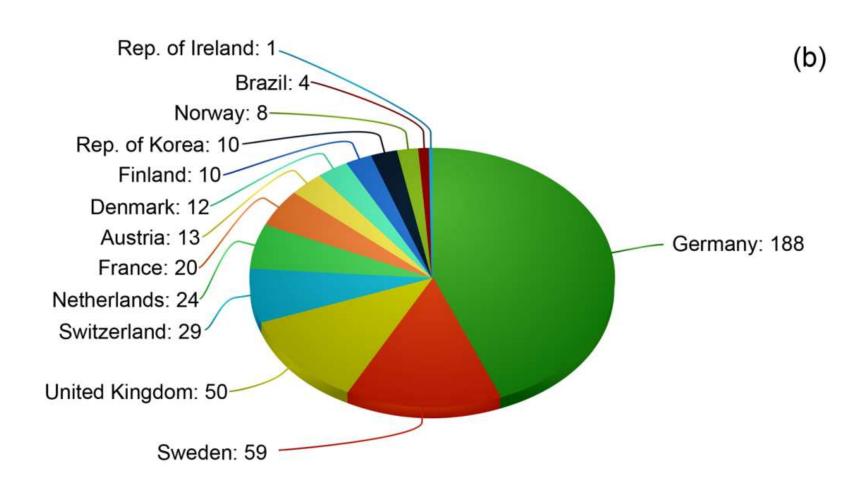
Applications?

Can be used as a **substitute for transport fuels**, to produce **combined heat and power** (CHP), **heat alone** or serve as **feedstock for the chemical sector**. It can be transported and stored in the facilities and infrastructure available for natural gas.



Source: IEA Bioenergy 2014, Joint Study by IEA Bioenergy Task 40 and Task 37

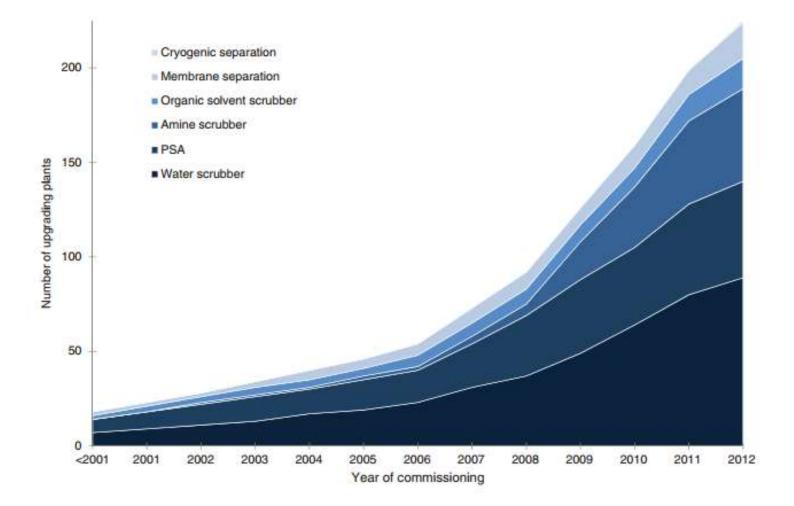
Biogas upgrading



IEA Bioenergy Task 37 as reported in Hoyer et al. (2016) and European Biogas Association Hoyer, K., Hulteberg, C., Svensson, M., Jernberg, J., Nørregard, Ø., 2016. Biogas Upgrading – Technical Review. ISBN: 978-91-7673-275-5.

Biogas upgrading





Source: Bauer, F., Persson, T., Hulteberg, C., & Tamm, D. (2013). Biogas upgrading-technology overview, comparison and perspectives for the future. *Biofuels, Bioproducts and Biorefining*, 7(5), 499-511.



Parameter	Water scrubber	PSA	Membrane (2-4 stages)	Chemical scrubber (amine)	Organic physical scrubber
CH ₄ in product gas	96 - 98 %	96 - 98 %	96 - 98 %	96 - 99 %	96 - 98 %
Availability	95 - 98%	95 - 98 %	95 - 98%	95 - 98%	95 - 98 %
Annual maintenance cost (% of investment cost)	2 - 3%	2 – 3 %	3 – 4 %	2 – 3 %	2 – 3 %
H ₂ S removal	Yes	External	External	External/Yes	External
H ₂ O removal	External	Yes	Yes	External	External
N ₂ and O ₂ separation	No	No/partly	Partly (O ₂)	No	No
Electricity consumption (product gas > 4 bar(g)) (kWh/Nm ³ raw biogas)	0.2 - 0.3	0.2 - 0.3	0.2 - 0.3	0.10 - 0.15	0.2 - 0.3
Heat (kWh/Nm ³ raw biogas)	None	None	None	0.5 – 0.6	Internal
Pure CO ₂	No	Yes	Yes	Yes	No

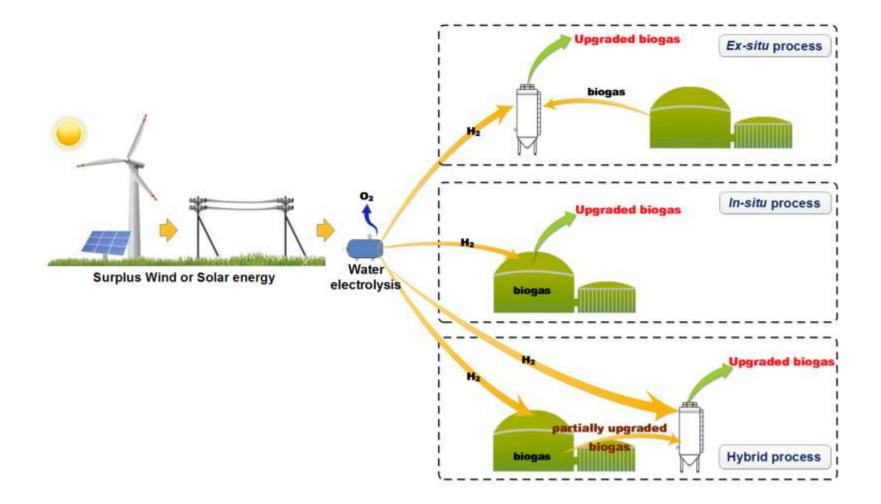
Source: Bauer F, Persson T, Hulteberg C, Tamm D (2013) Biogas upgrading – Review of commercial technologies. Swedisch Gas Technology Centre (SGC), Malmö

Surplus Energy/Energy Storage?

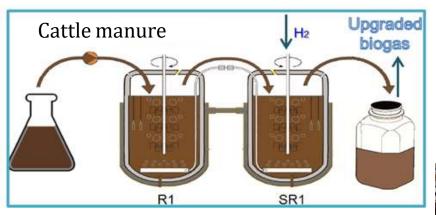


Biological biogas upgrading



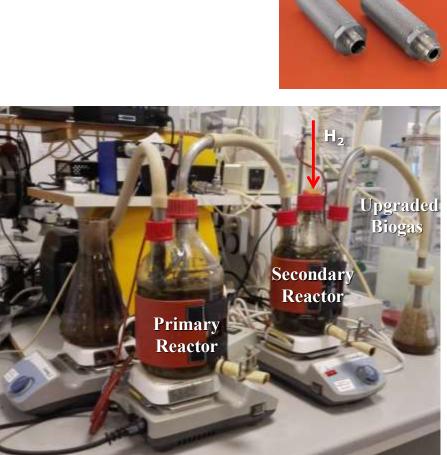


Example 1: Serial configuration



Reactor	Working volume (L)	Temp. (°C)	HRT (days)
R1	1.5	35±1	25
SR1	2.0	35±1	33
R2	1.5	55±1	15
SR2	2.0	55±1	20

Technical challenge: limitations in injection of H₂ using metal diffusers

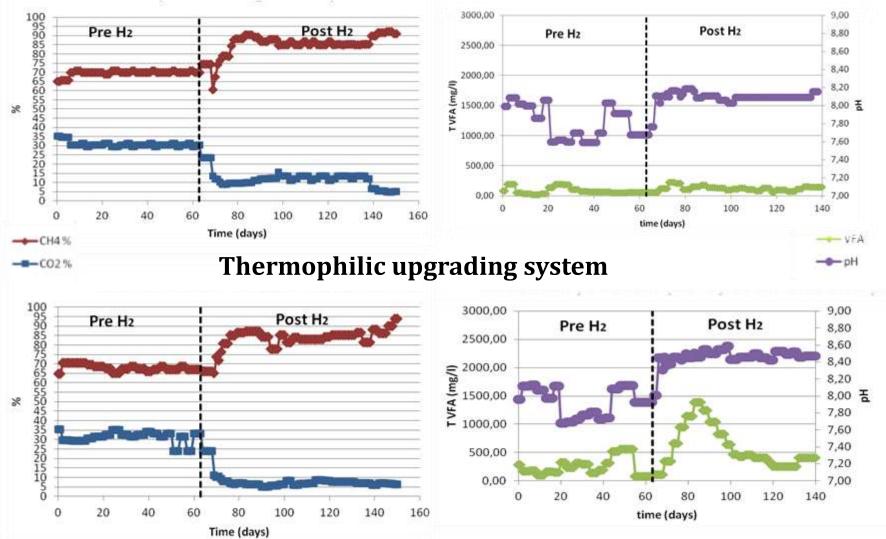


Bassani *et al 2015 Environ. Sci. Technol.*

Example 1: Serial configuration



Mesophilic upgrading system



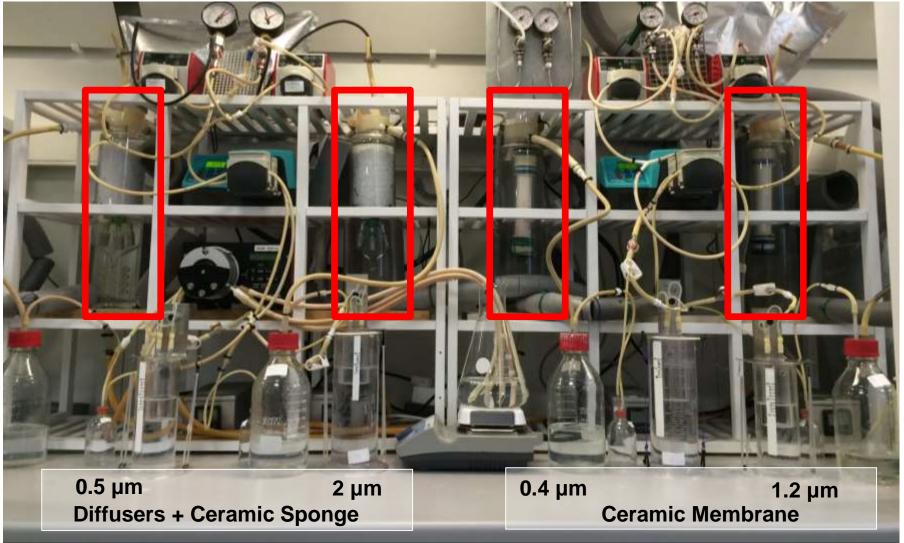
Bassani *et al 2015* Environ. Sci. Technol.

DTU Environment, Technical University of Denmark

Example 2: Ex-situ biogas upgrade



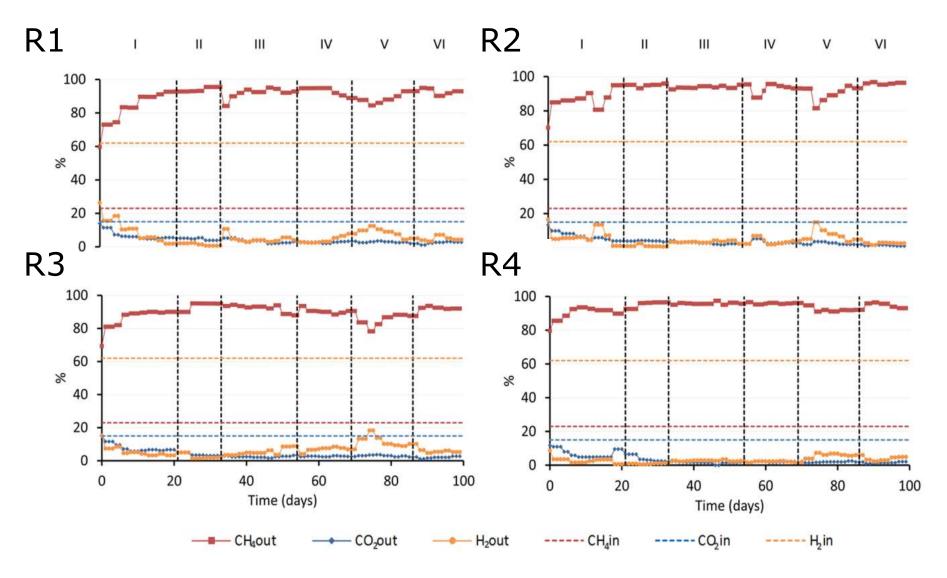
62% H₂ - 23% CH₄ - 15% CO₂



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Bassani *et al 2017 Bioresour. Technol.*

Example 2: Ex-situ biogas upgrade



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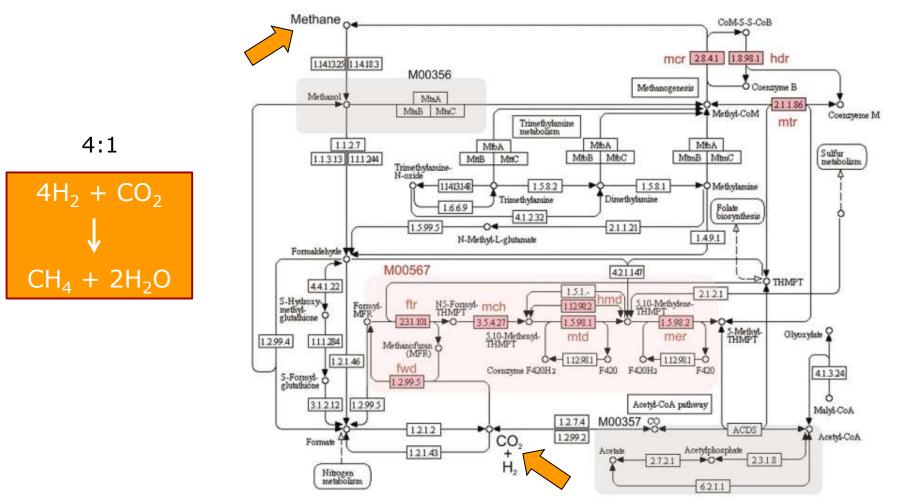
Bassani *et al 2017 Bioresour. Technol.*

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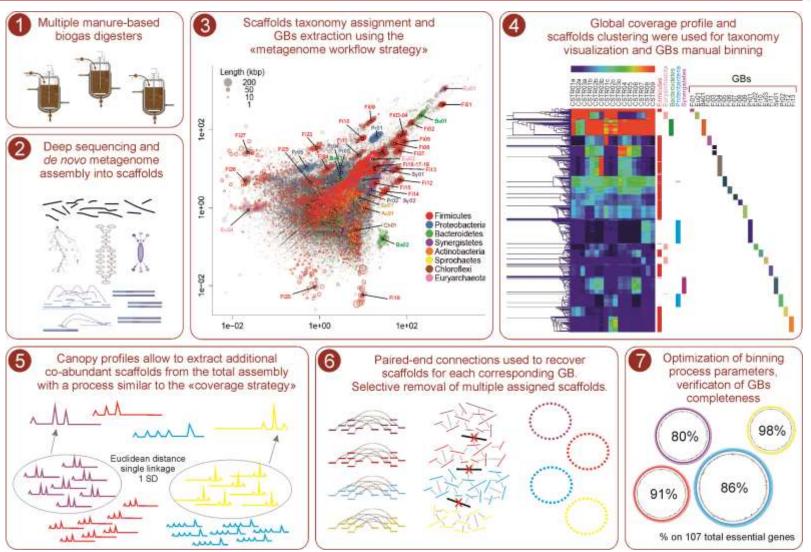
Biological biogas upgrading



In upgrading anaerobic digestion system the injected exogenous H_2 is coupled with CO_2 and converted into CH_4 by the action of **hydrogenotrophic methanogens**



Metagenomic approach: binning



biogasmicrobiome.com

Campanaro et al. 2016 Biotechnol. Biofuels

Microbial upgrading taxonomy



Comparison of high-resolution microbial trees with **phylogeny and putative taxonomy**, obtained using 400 broadly conserved proteins with PhyloPhlAn software.

> On the right the **106** Population Genomes (PGs) identified in standard thermophilic anaerobic digesters.

> > On the left the **236** PGs binned from mesophilic and thermophilic **biogas upgrading** reactors.

biogasmicrobiome.com

Firmicute

Treu *et al. 2016 Bioresour. Technol.*

Microbial upgrading taxonomy



Some taxa were found independently from reactors operational conditions, for example several **recurrent phylotypes** are:

Methanoculleus, Methanothermobacter, Synthrophomonas and Proteobacteria.

	Cor microb		Thermo react	-	Upgrading reactors			
Taxon	GBs	%	GBs	%	GBs	%		
Firmicutes	56	36%	13	8%	85	55%		
Syntrophomonadaceae	24	50%	0	0%	24	50%		
Bacteroidetes	5	17%	1	3%	24	80%		
Proteobacteria	4	14%	6	21%	19	66%		
Synergistetes	3	30%	3	30%	4	40%		
Spirochaetes	1	13%	1	13%	6	75%		
Actinobacteria	0	0%	1	17%	5	83%		
Chloroflexi	0	0%	0	0%	6	100%		
Euryarchaeota	4	67%	1	17%	1	17%		
Tenericutes (Firmicutes)	3	50%	1	17%	2	33%		
Verrucomicrobia	0	0%	0	0%	3	100%		
Thermotogae	1	50%	1	50%	0	0%		
Fibrobactere	0	0%	0	0%	1	100%		
Acidobacteria	0	0%	0	0%	1	100%		
Chlamydiae	0	0%	0	0%	1	100%		
Planctomycetes	0	0%	0	0%	1	100%		
TM7	0	0%	1	100%	0	0%		

Composition of archaeal community was found to be **resilient**, while bacterial community was more **diverse** due to higher functional variability.

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Treu L. *et al.* 2016 *Bioresour. Technol.*

Microbial upgrading populations



Mesophilic community

Thermophilic community

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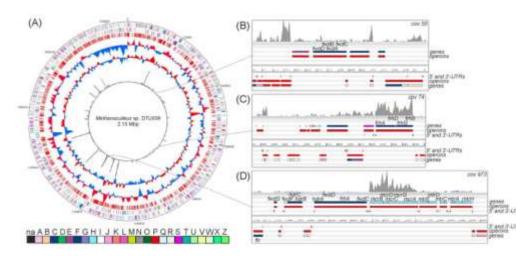
Microbial **changes in abundance** before (light) and after (dark) H_2 addition are represented as circles with areas proportional to **genome coverage**

Microbial upgrading populations



					Mesophilic		Thermophilic				
Phylum	Population genome	Таха	16S rRNA gene	Before H ₂	After H ₂	fold change	Before H ₂	After H ₂	fold change		
Bacteroidetes	DTU134	Rikenellaceae	A. shahii (86%)	38.8	156.6	4.04	1.0	0.5	2.24		
Bacteroidetes	DTU002	Bacteroidales	P. propionicigenes (85%)	84.0	70.8		2.0	0.4	4.40		
Firmicutes	DTU175	Thermoanaerobacteraceae	M. thermoacetica (87%)	34.3	98.4	2.87	0.1	0.3	5.47		
Bacteroidetes	DTU135	Bacteroidales	O. hongkongensis (86%)	27.0	19.3	1.40	1.8	0.2	9.38		
Bacteroidetes	DTU136	Bacteroidaceae	B. clarus (87%)	13.4	29.9	2.23	0.7	0.2	3.54		
Firmicutes	DTU220	Syntrophomonadaceae	nd	3.9	32.6	8.41	1.6	2.1	1.37		
Firmicutes	DTU224	Erysipelotrichaceae	nd	22.1	4.2	5.26	0.0	0.0	1.88		
Bacteroidetes	DTU137	Porphyromonadaceae	T. forsythia (83%)	3.8	20.1	5.34	0.0	0.0	1.15		
Acidobacteria	DTU170	Acidobacteriales	A. capsulatum (86%)	7.8	14.3	1.82	0.0	0.0	1.83		
Firmicutes	DTU230	Clostridiales	nd	9.5	11.6		0.1	0.5	7.30		
Firmicutes	DTU246	Clostridiales	nd	14.7	3.0	4.92	0.4	0.4	1.14		
Chloroflexi	DTU159	Chloroflexi	nd	10.6	3.9	2.74	0.2	0.1	3.71		
Firmicutes	DTU010	Syntrophomonadaceae	nd	6.8	3.1	2.20	286.2	461.8	1.61		
Bacteroidetes	DTU001	Alistipes	nd	0.3	0.1	4.13	158.2	19.5	8.10		
Firmicutes	DTU011	Thermoanaerobacteraceae	Th. toyohensis (86%)	0.9	1.3	1.49	68.1	96.0	1.41		
Euryarchaeota	DTU006 (Methanoculleus	M. marisnigri (87%)	0.2	0.3	1.92	40.4	50.8			
Firmicutes	DTU012	Syntrophomonadaceae	nd	0.0	0.0	1.89	24.9	34.1	1.37		
Firmicutes	DTU014	Clostridiales	D. carboxydivorans (85%)	0.0	0.0	1.05	17.3	31.2	1.81		
Firmicutes	DTU013	Clostridiaceae	nd	0.0	0.0	1.09	16.4	30.7	1.87		
Firmicutes	DTU183	Syntrophomonadaceae	nd	0.0	0.0	1.45	19.4	26.1	1.34		
Firmicutes	DTU176	Syntrophomonadaceae	nd	0.0	0.0	1.11	9.5	14.7	1.55		
Firmicutes	DTU029	Halothermothrix	nd	0.0	0.0	1.26	5.6	13.1	2.34		
Firmicutes	DTU021	Syntrophomonadaceae	nd	0.1	0.1	2.08	9.5	9.0			
Firmicutes	DTU030	Syntrophomonadaceae	nd	0.0	0.0	1.98	8.6	9.3			
Thermotogae	DTU111	Defluviitoga tunisiensis	D. tunisiensis (98%)	0.0	0.0	1.73	12.5	4.6	2.72		

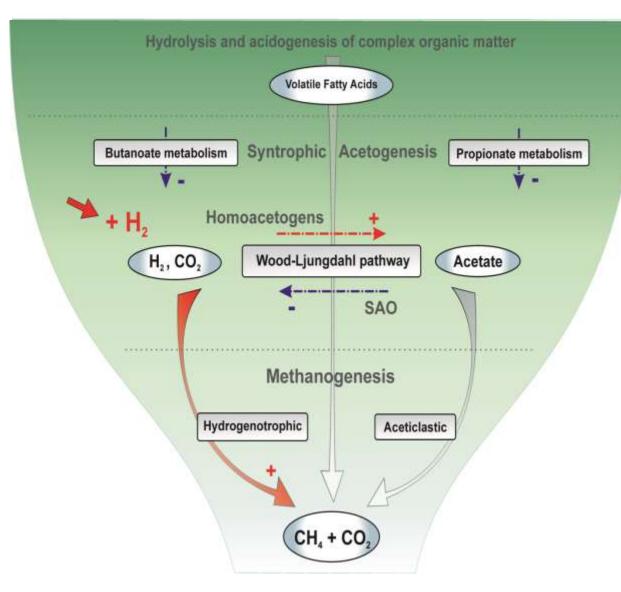
Novel microbial species



Candidatus Methanoculleus thermohydrogenotrophicum

GENOME CHARCATERISTICS									
Genome size [bp]	2.15 Mbp								
GC content	59.20%								
Scaffold N50 [bp]	17,178								
Number of contigs	503								
Number of protein-encoding genes	2,297								
Total number of essential genes	32								
Estimated completeness % (CheckM)	92.70%								
Estimated contamination level % (CheckM)	2.30%								

Anaerobic digestion: the funnel concept



 Functional classification

- ✓ Generalist GBs
- ✓ Specialist GBs

Some microbes are **favored** by H₂ addition some other are **inhibited**, at all levels of the funnel, but in particular at the lower levels

Effect of H₂ on syntrophic bacteria



Tentative functional reconstruction of specific population genomes with different responses to H_2 exposure

		Meso	philic	Therm	ophilic						
Population genome	Таха	Before H ₂	After H ₂	Before H ₂	After H ₂	Propanoate metabolism			Fatty acid degradation	W-L pathway	Fdh
DTU232	Syntrophomonadaceae	9.30	0.37	0.01	0.00	15	24	28	10	8	1
DTU204	Syntrophomonadaceae	2.42	0.31	0.14	0.10	8	12	22	18	9	4
DTU223	Syntrophomonadaceae	1.26	1.12	1.52	0.76	2	4	18	0	8	3
DTU183	Syntrophomonadaceae	0.01	0.00	19.41	26.06	14	20	32	14	8	3
DTU077	Syntrophomonadaceae	0.01	0.01	0.45	7.39	4	14	23	14	10	4
DTU122	Syntrophomonadaceae	0.00	0.00	0.49	2.19	8	1	30	1	8	5
DTU063	Tepidanaerobacter	0.01	0.00	8.34	1.66	15	10	33	0	8	
DTU021	Syntrophomonadaceae	0.14	0.07	9.47	8.97	10	22	30	21	10	3
DTU236	Syntrophomonadaceae	0.01	0.06	1.17	1.83	18	24	29	5	10	2
DTU093	Syntrophomonadaceae	0.01	0.01	1.23	1.81	16	29	39	2	9	1
DTU245	Syntrophomonadaceae	4.65	8.77	0.00	0.01	20	17	32	9		
DTU190	Syntrophomonadaceae	0.11	0.06	0.46	2.26	24	45	34	25		8
DTU052	Syntrophomonadaceae	0.13	0.09	1.59	3.92	19	35	34	38		9

On average >92% completeness and < 4% contamination

Conclusions



- Biological biogas upgrading is a feasible biomethanation
 technology leading to more than 96% CH₄ content in the final gas
- Independently form the H₂ addition distinct microbial
 communities were shaped due to the operational temperature
- ✓ H₂ addition enriched the hydrogenotrophic methanogenic culture and promoted the formation of syntrophic interactions
- Novel methanogen species was identified and was proposed as
 Candidatus Methanoculleus thermohydrogenotrophicum
- Correlation between syntrophs response to H₂ addition and their metabolic pathways was established

Teamwork





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Irini Angelidaki Professor DTU-ENV



Università degli Studi di Padova

Department of Biology

eBio



Ilaria Bassani PhD student



Panagiotis Tsapekos PostDoc



Xinyu Zhu PhD student



Thank you for your attention



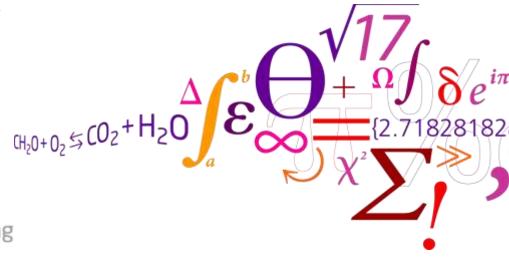
InnovationsFonden

FORSKNING, TEKNOLOGI & VÆKST I DANMARK

SYMBIO – Integration of biomass and wind power for biogas enhancement and upgrading via hydrogen assisted anaerobic digestion -0603-00525B

ENERGINET

BioUpgrade– Ex-situ biogas upgrading through biologically mediated CO2 reduction, 12465



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