Optimized microbial conversion of bituminous coal to methane for *in situ* and ex situ applications

Kick off meeting

12-09-2014

Outline

- Overall objectives
- Proposed tasks
- Background
- Results previous to the DOE project
- Task 1.0
- Task 2.0

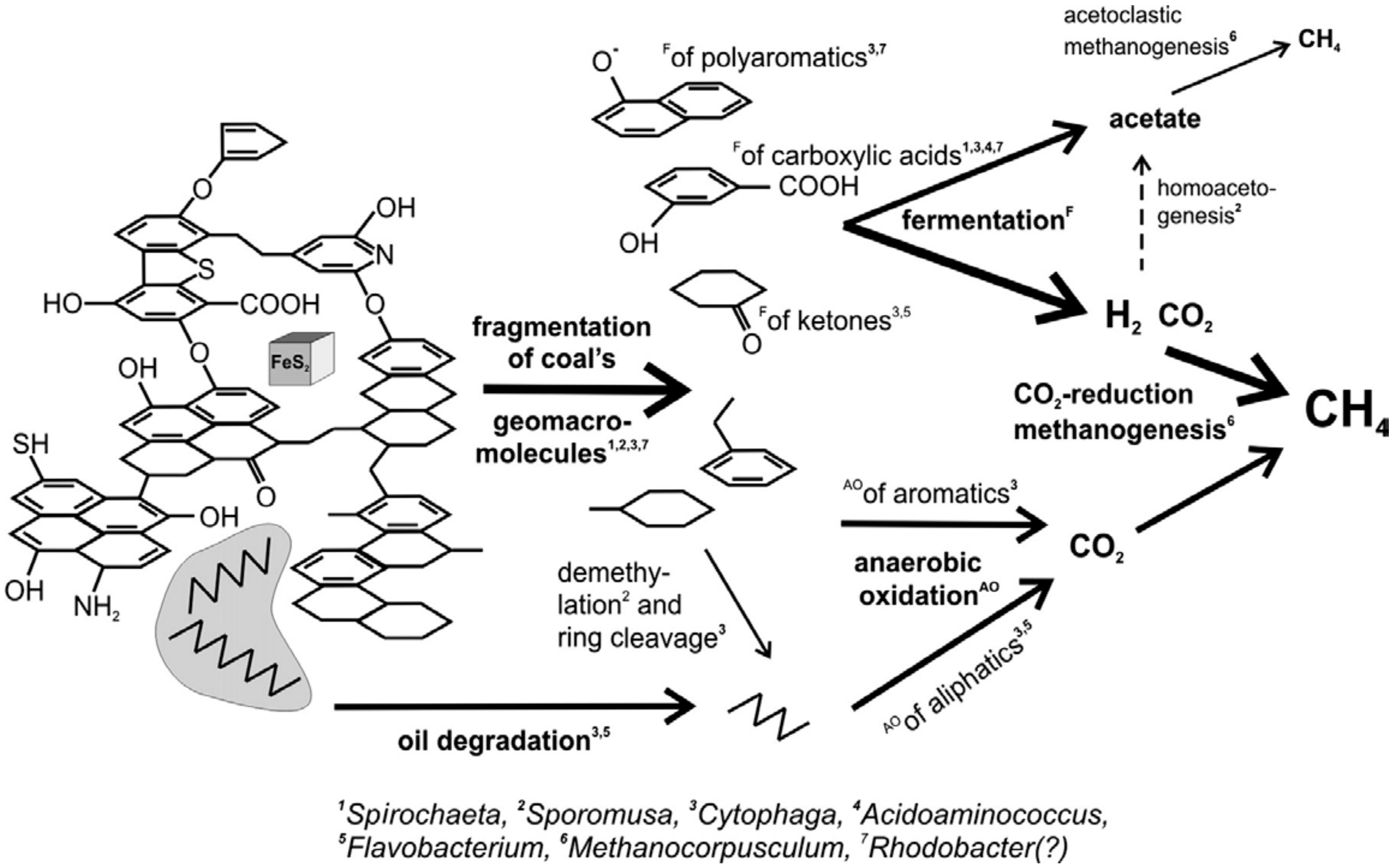
Objectives

- 1) optimize methane production from bituminous coal through biological coal conversion (BCC)
- 2) demonstrate feasibility of the developed technology for *ex situ* and *in situ* applications.

Tasks

- Task 1 - Project Management, Planning and Reporting
- Task 2.0: Simplify the composition of the nutrient medium
- Task 3.0: Investigate individual and interactive effects from different parameters
- Task 4.0: Maximize methane productivity in a fed-batch system
- Task 5.0: Bioconversion under pressurized conditions
- Task 6.0: Ecological Considerations of the Proposed Process
- Task 7.0: Economic Analysis of the Proposed Process

Proposed pathways: from coal to methane



$$CH_3COOH \rightarrow CH_4 + CO_2$$

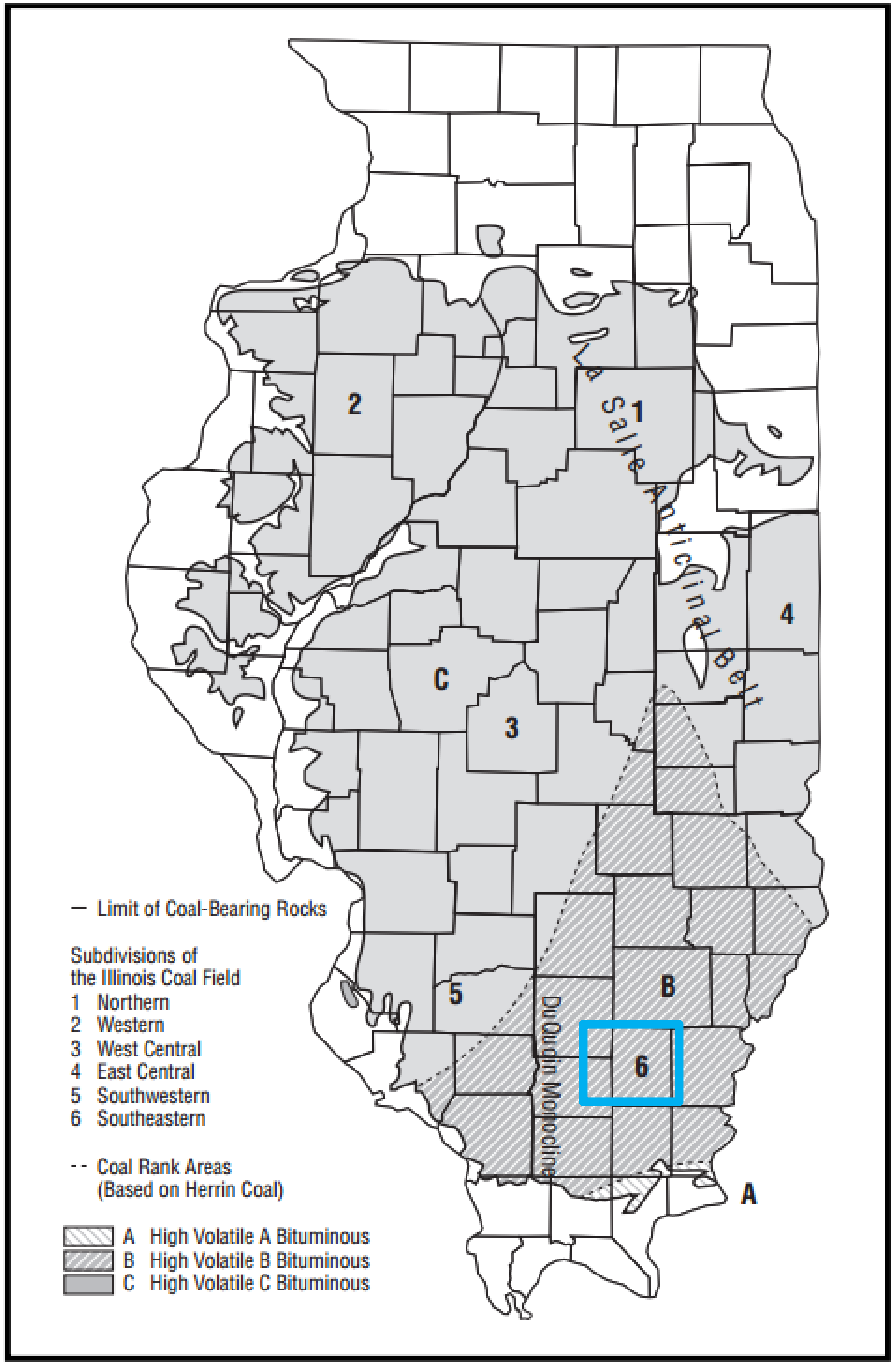
$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

Strąpoć D et al. Appl. Environ. Microbiol. 2008;74:2424-2432

Previous results

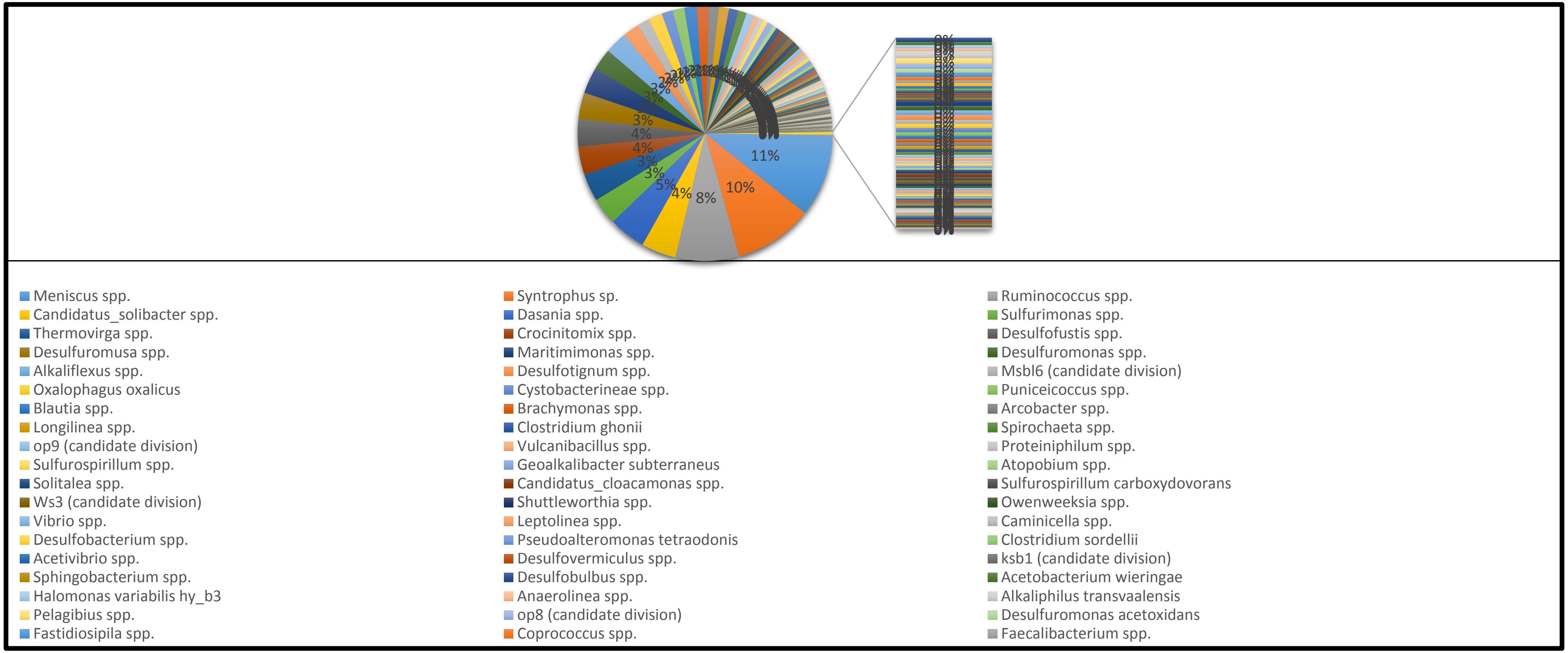
- Microbial structure of the original microbial community in the formation water
- Microbial population of the adapted consortium
- Methane production from bituminous coal
- Identification of extracellular proteins
- Properties of coal residue after bioconversion

Sampling sites



http://isgs.illinois.edu/sites/isgs/files/files/coal-maps/Illinois-coalgeology.pdf

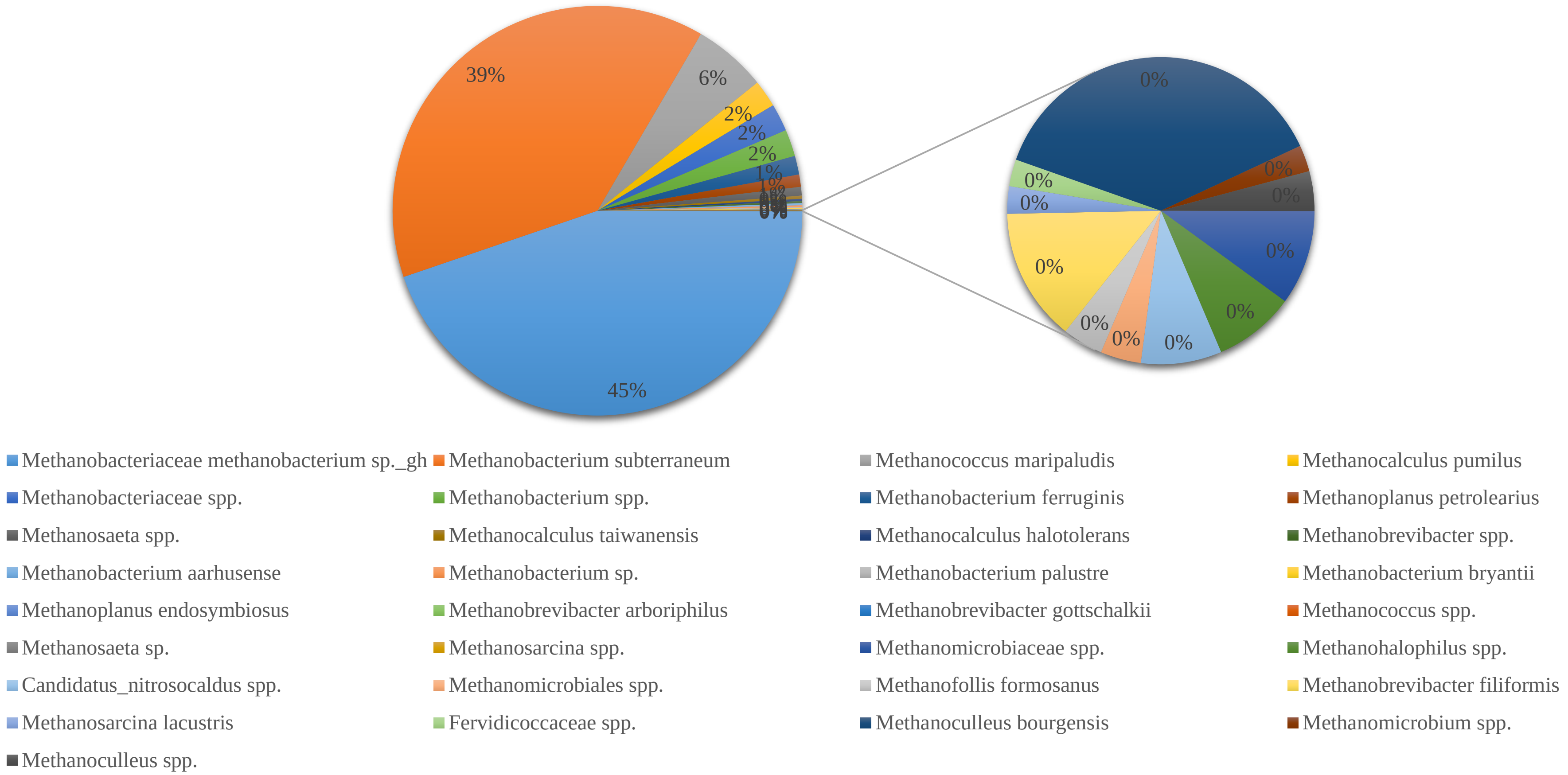
Bacteria species in the formation water



231 bacterial species

The top species can degrade polymers, utilize benzoate, fatty acids, sugars for growth, reduce sulfate.

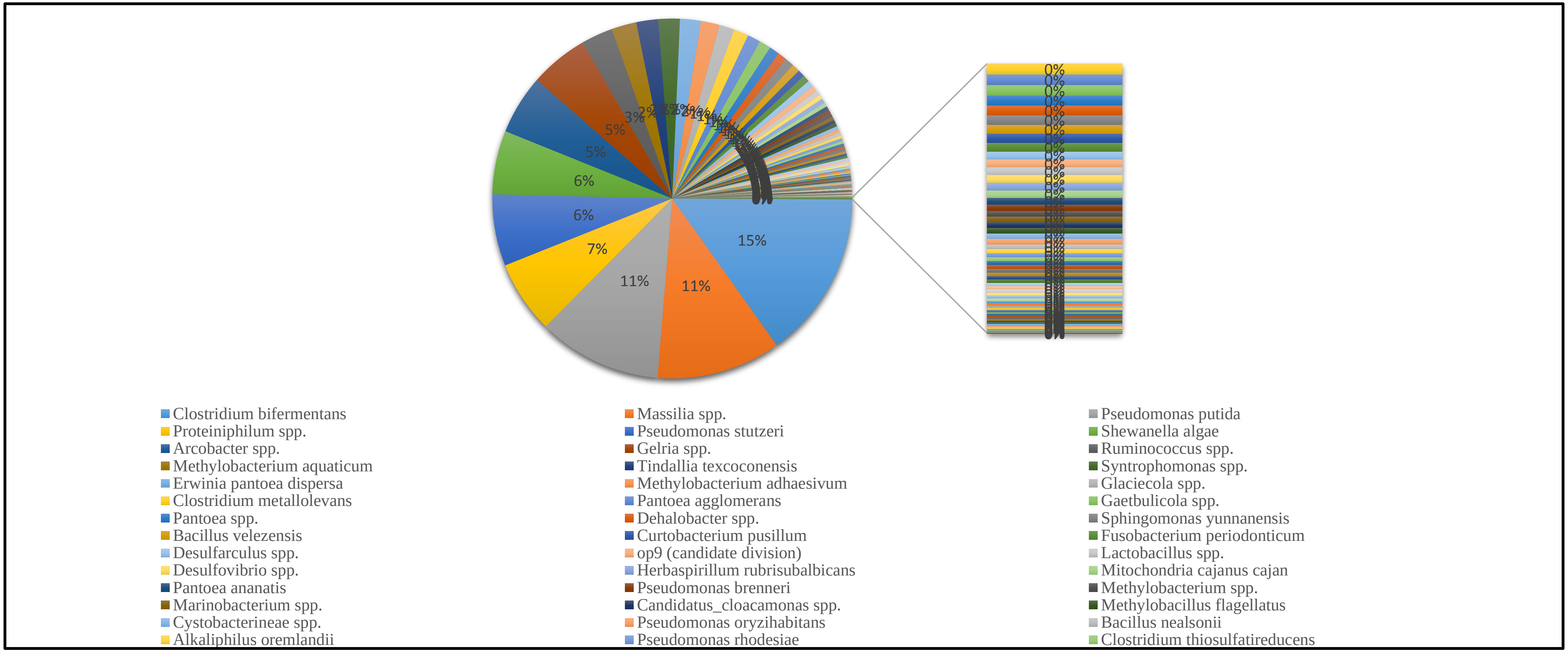
Archaea species in the formation water



33 archaea species

The majority (89.8%) of archaea belonged to the order of Methanobacteriales

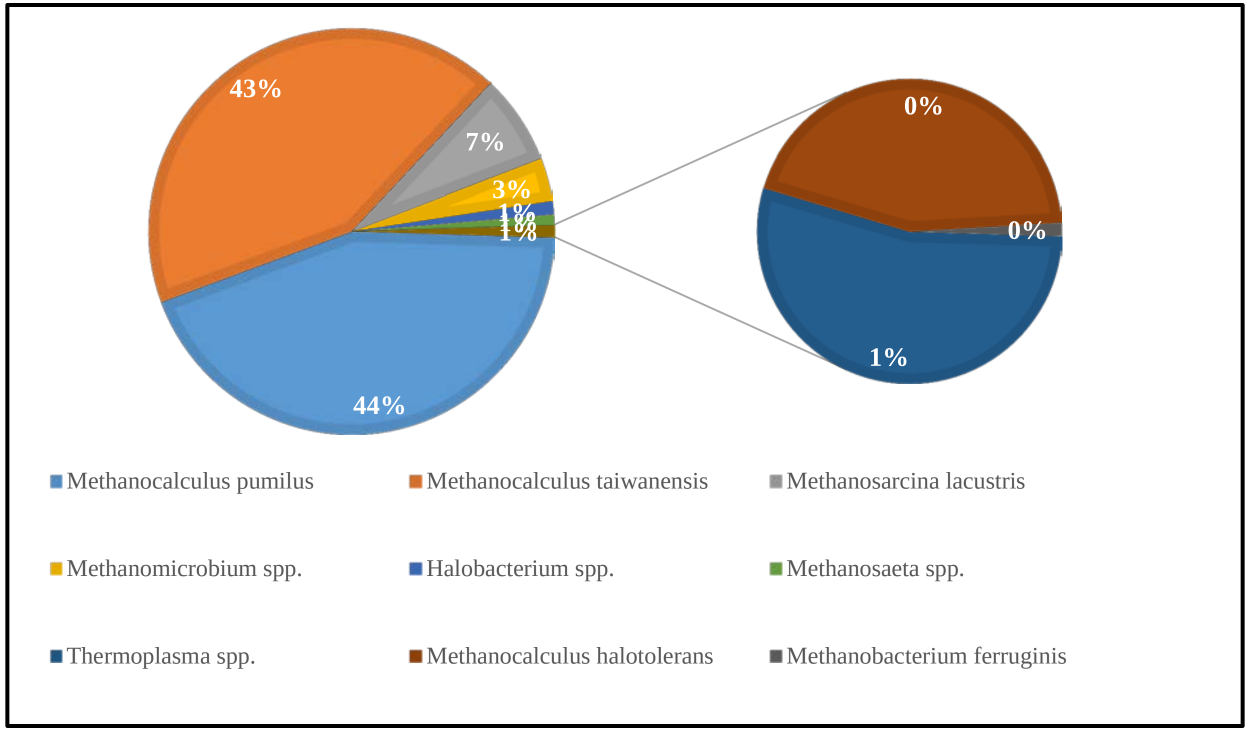
Bacterial species in the adapted consortium



185 species

Dominant ones in the original community became minor.

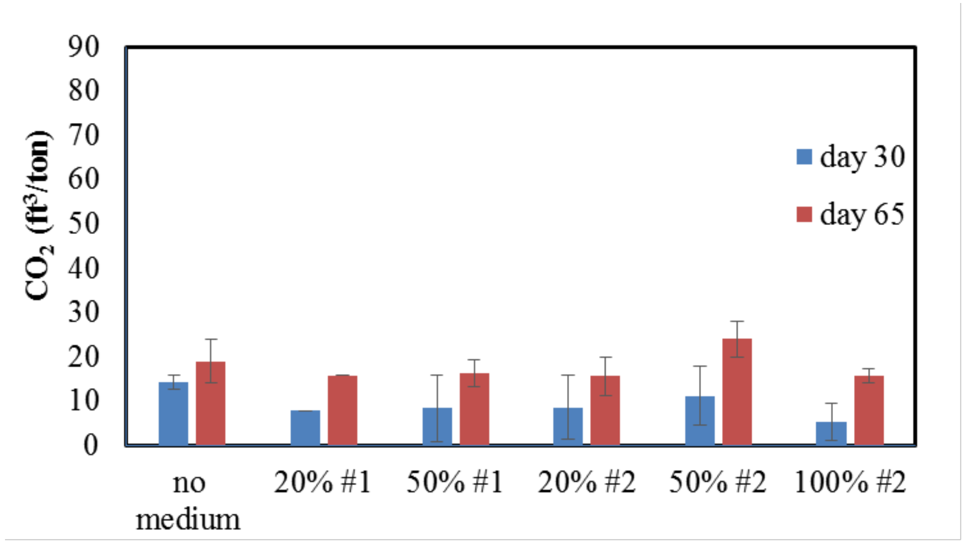
Archaea species in the adapted consortium

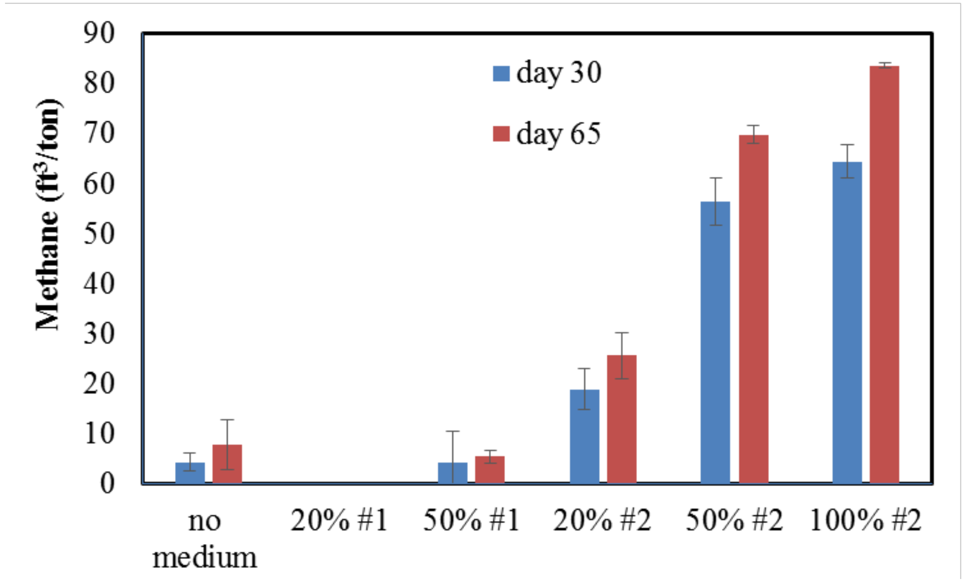


nine species

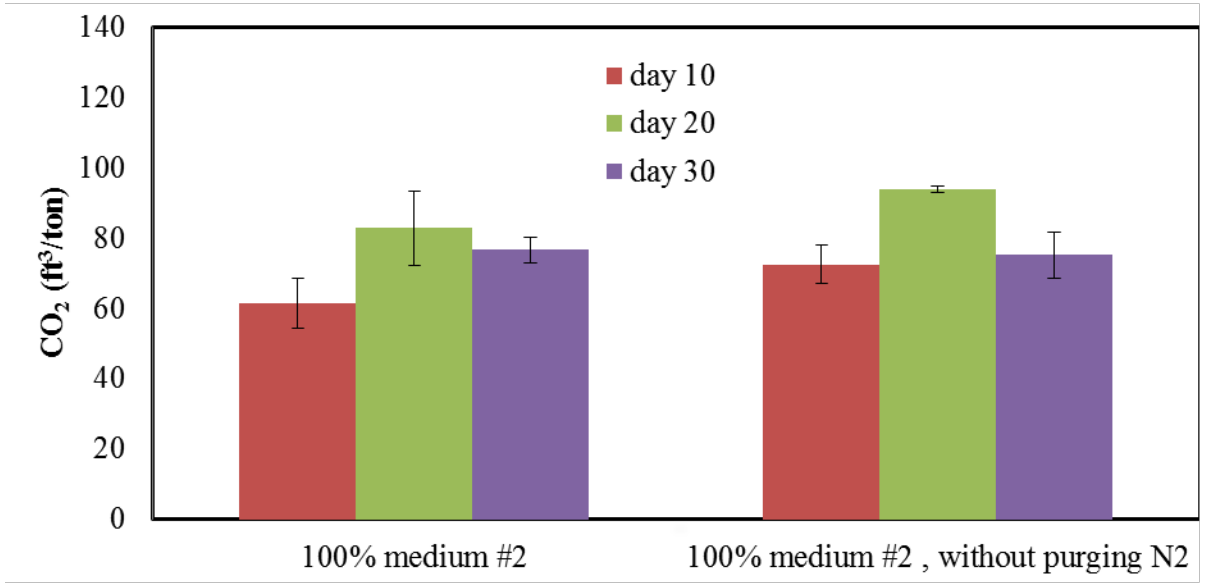
Minor order of *Methanomicrobiales* became the dominant one (90.4%).

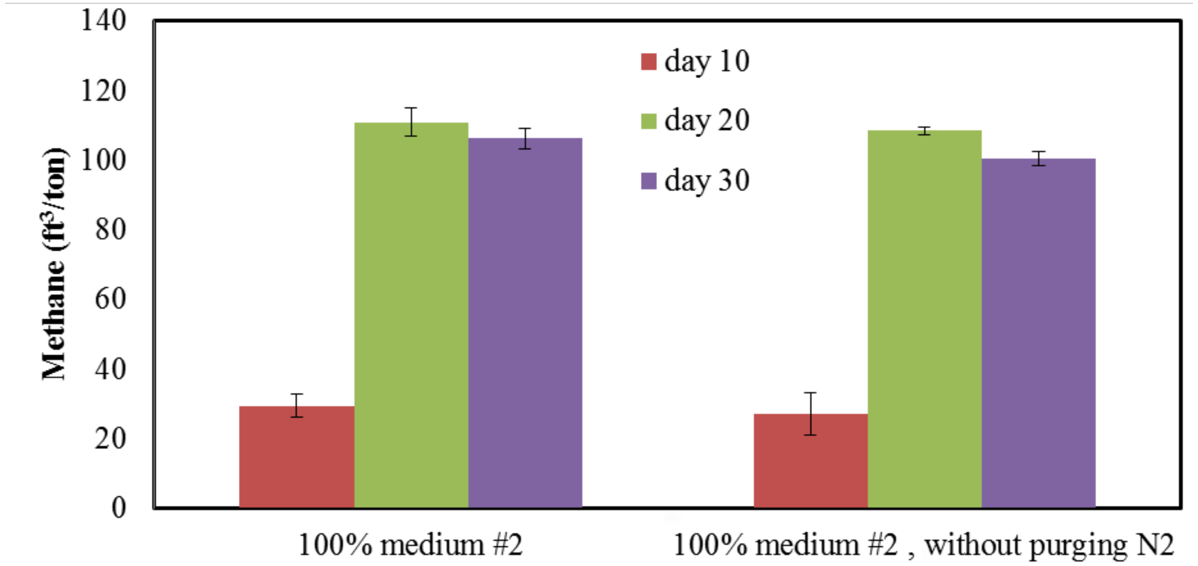
Methane/CO_2 production: comparison among different solution





Methane/CO_2 production: with and without purging

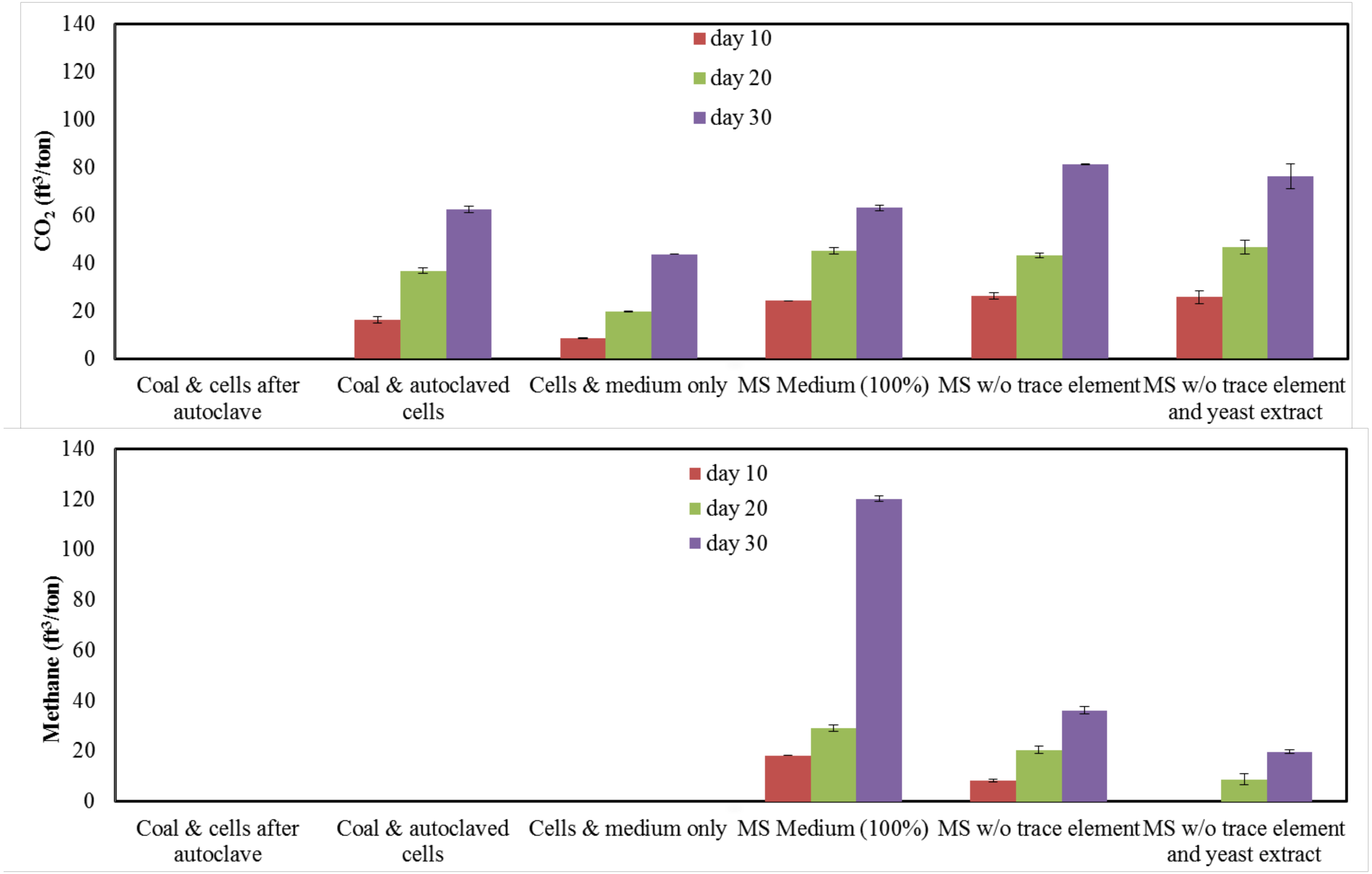




Results

- Medium #2 is better than medium #1 in terms of enhancing methane yield.
- After adding acclimated microbial consortium to coal in medium #2, purging with N_2 is not necessary to create an anaerobic environment.
- The acclimated microbial consortium gave a methane yield of 5.6 ft^3/ton/day during the first 20 days, which is higher than 1.3 ft^3/ton/day in 65 days through the original microbial community.

Methane/CO_2 production: comparison with various controls



Results

- The coal sample we used contain microbial strains. But they are unable to produce methane.
- The acclimated microbial consortium can generate CO_2 from carbon sources (yeast extract, peptone) in the nutrient solution or medium. But they cannot produce methane from these carbons.
- All methane we observed is from coal degradation.
- Using coal (40-70 mesh), methane production rate is 4.0 ft^3/ton/day, which is lower than 5.6 ft^3/ton/day from finer coal (< 40 mesh).

Comparison with other reported results

Coal source	Coal rank	Source of microbial community	Methane production rate ft3/ton/day	Temperature ©	Shaking	Test duration (days)	Particle size (um)	Reference
Powder river basin	Subbituminous B	Well water	2.7	22	100 rpm	26	250-600	Green et al., 2008
Texas	Subbituminous	Wetland sediment	0.8	22	No	70	2000-10000	Jones et al., 2010
Texas	Lignite	Wood eating termite	424-600	37	No		44-595	Harding et al., 1993
Utah	Bituminous	waste coal	73.1	23	No	30	< 74	Opara et al., 2012
	coal waste		43.8a					
	Lignite		40.2					
Australia	Subbituminous	Formation water	30	37	50 rpm		300-600	Papendick, 2011
Pocahontas No. 3	Low volatile bituminous	Formation water	2.1b	22	No	60	NA	Fallgren et al.., 2013
Pittsburgh NO. 8	High volatile bituminous	Formation water	1.2b	22				
Illlinois	Bituminous	Formation water	5.6	28	No	20	<420	This study

Enzymes involved in methane formation

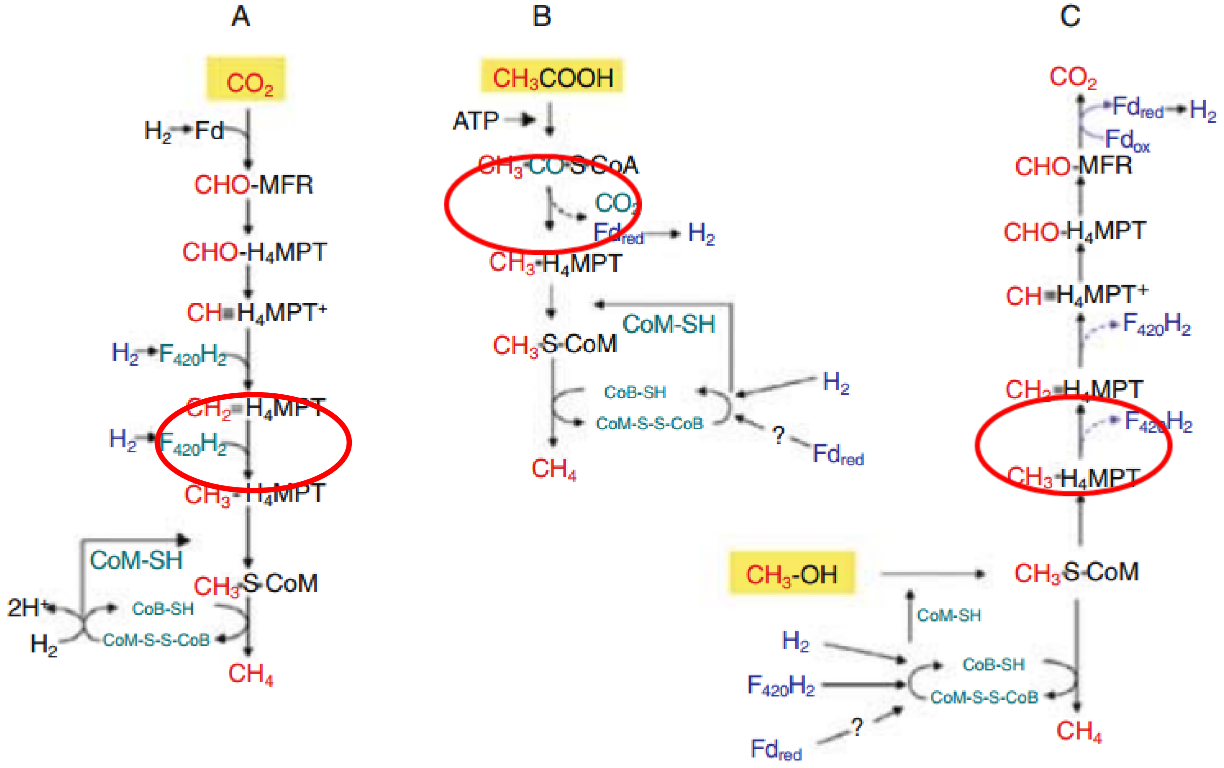
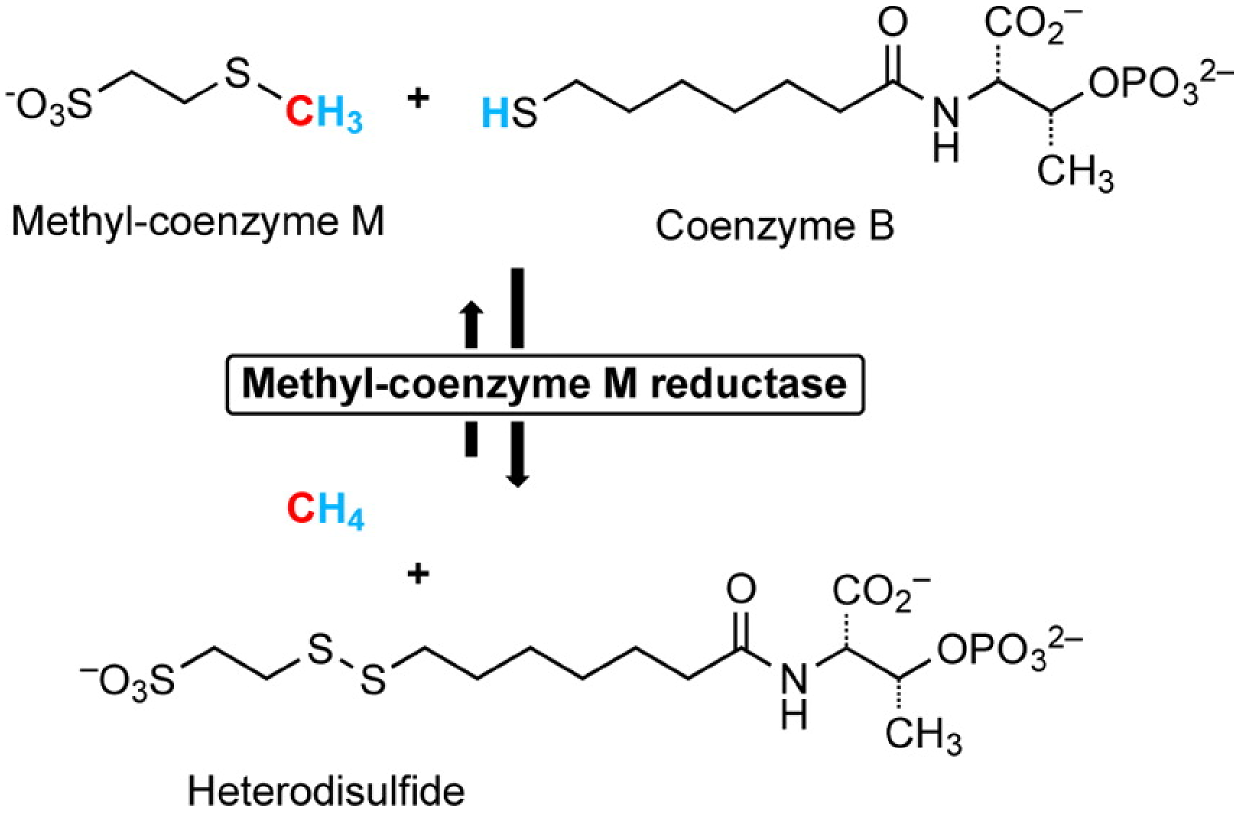


Fig. 2. Scheme of methanogenesis from H_2/CO_2 (A), acetate(B) and methanol (C). Methyl-coenzyme M (CH_3-S-CoM) is a central intermediate in all three pathways. It is converted to methane and the heterodisulfide of coenzyme M and coenzyme B (CoM-S-S-CoB). CoM-S-S-CoB thus generated functions as the terminal electron acceptor of different respiratory chains. H_2 and reduced coenzyme F_{420} ($F_{420}H_2$) have been identified as electron donors for the reduction of CoM-S-S-CoB. The unknown mechanism of electron transfer from the reduced ferredoxin ($Fd_{(red)}^-$) to CoM-S-S-CoB in acetate- and methanol metabolism is symbolized by a question mark. The role of H_2 as an intermediate of this reaction is discussed below (see Fig. 14). Abbreviations: CHO-FMR, *N*-formylmethanofuran; CHO-H_4MPT, N^5-formyltetrahydromethanopterin; CH=H_4MPT^+, N^5,N^{10}-methenyl-tetrahydromethanopterin; CH_2=H_4MPT, N^5,N^{10}-methylene-tetrahydromethanopterin; and CH_3-H_4MPT, N^5-methyl-tetrahydromethanopterin. For structures of the coenzymes, see Figs. 3, 7 and 9. For simplicity, only tetrahydromethanopterin (H_4MPT) is shown. For other methanopterin derivatives, see Fig. 9.

Physiology and Biochemistry of the Methane-Producing Archaea, Hedderich and Whitman, 2006. The Prokaryotes. 1050-1079.



Published in: Silvan Scheller; Meike Goenrich; Rudolf K. Thauer; Bernhard Jaun; *J. Am. Chem. Soc.* **2013,** 135, 14975-14984.
DOI: 10.1021/ja406485z

Category	**Protein name**	**Function**	**# of protein family**	**Abundance**
Substrate transport			***52***	
	Outer membrane proteins	Substrate binding, transport	14	2.37
	ABC transporter substrate-binding	Transport amino acids, sugars, phosphonate	18	0.58
	Porin	Channels for specific molecule transport	3	0.32
	C4-dicarboxylate ABC transporter	Transport malate, succinate and fumarate across the cytoplasmic membrane	3	0.14
	Starch-binding protein	Bind and transport starch	1	0.07
	Collagen-binding protein	Bind and transport collagen	1	0.04
	Others	Molecular chaperone, receptor, etc	8	Various
Cellular metabolism			***36***	
	Glutamate dehydrogenase	Convert glutamate to oxoglutarate. NH_4^+ is released through the reaction	5	1.38
	Flagellin protein	Structural protein that forms the major portion of flagellar filaments	5	0.39
	Transcriptional regulator	Regulate transcription from DNA to RNA	5	0.39
	Cytochrome c	Coupling factor for hydrogenase	2	0.16
	Others	ATP synthase, DNA polymerase, etc.	19	Various
Substrate utilization			***24***	
	Iron hydrogenase	Catalyze the reversible reaction of hydrogen to hydrogen ion	2	0.48
	Glucokinase	Phosphorylate glucose to glucose-6-phosphate	1	0.13
	Sulfite reductase	Reduce sulfite	3	0.11
	Rhamnulokinase	Phosphorylate rhamnulose to rhamnulose 1-phosphate	1	0.08
	Glycoside hydrolase	Assist in the hydrolysis of glycosidic bonds in complex sugars	1	0.05
	Others	Various	16	Various
Oxidative stress			***10***	
	Rubrerythrin	May reduce hydrogen peroxide	1	1.27
	Ssuperoxide dismutase	Reduce superoxide	3	0.22
	Peroxidase	Reduce peroxide	1	0.21
	Others	Oxidoreductase	5	Various
Hypothetical proteins	Various	Unknown	***37***	Various

Task 4: Identification of enzymes secreted by bacteria

Category	Protein name	Function	# of protein family	Abundance
Substrate transport			***52***	
	Outer membrane proteins	Substrate binding, transport	14	2.37
	ABC transporter substrate-binding	Transport amino acids, sugars, phosphonate	18	0.58
	Porin	Channels for specific molecule transport	3	0.32
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	Starch-binding protein	Bind and transport starch	1	0.07
	Collagen-binding protein	Bind and transport collagen	1	0.04
	Others	Molecular chaperone, receptor, etc	8	Various
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	Others	ATP synthase, DNA polymerase, etc.	19	Various

Task 4: Identification of enzymes secreted by bacteria-conc.

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	Sulfite reductase	Reduce sulfite	3	0.11
	Rhamnulokinase	Phosphorylate rhamnulose to rhamnulose 1-phosphate	1	0.08
	Glycoside hydrolase	Assist in the hydrolysis of glycosidic bonds in complex sugars	1	0.05
	Others	Various	16	Various
Oxidative stress			***10***	
	Rubrerythrin	May reduce hydrogen peroxide	1	1.27
	Ssuperoxide dismutase	Reduce superoxide	3	0.22
	Peroxidase	Reduce peroxide	1	0.21
	Others	Oxidoreductase	5	Various
Hypothetical proteins	Various	Unknown	***37***	Various

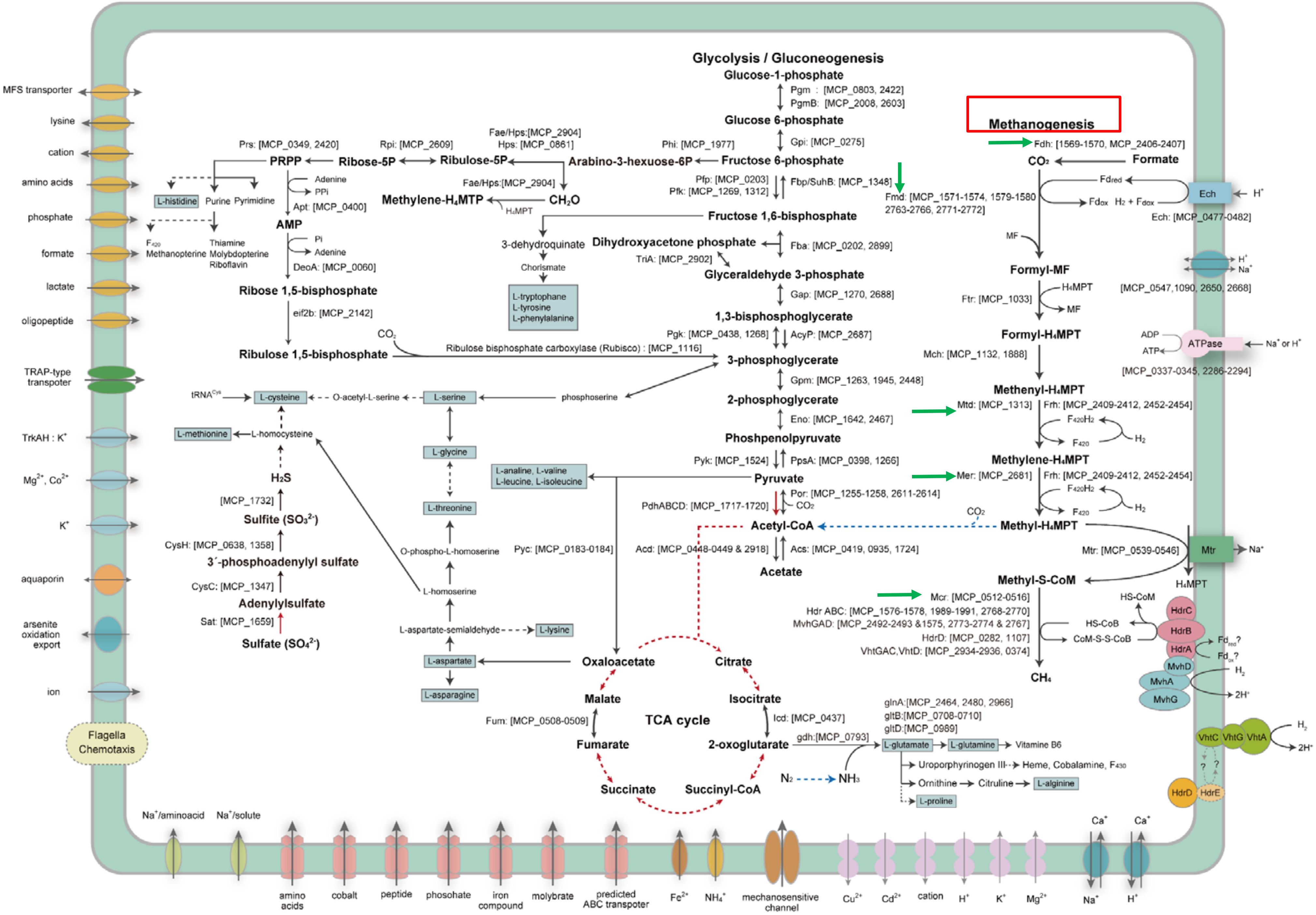
Category	Protein name	Function	# of protein family	Abundance
Related to methane production			*40*	
	Methyl-coenzyme M reductase (Mcr)	Catalyze methane formation for all methanogens	7	5.09
	Methanol-5-hydroxybenzimidazolylcobamide methyltransferase	Reduce methanol to methane	5	0.56
	Methylene tetrahydromethanopterin dehydrogenase (Mtd)	H_2 forming for CO_2 reduction	2	0.75
	Methylene tetrahydromethanopterin reducatase (Mer)	The fifth enzyme in CO_2 reduction	3	0.13
	Formyl-methanofuran dehydrogenase (Fmd)	The first enzyme in CO_2 reduction	2	0.09
	Formate dehydrogenase (Fdh)	Enable cells to utililze formate as electron donor	2	0.12
	Acetyl-CoA synthase	Allow growth on acetate as sole source of carbon and energy	3	0.18
	Disulfide reductase	Catalyze the final step of methane production	1	0.06
	Phosphotransacetylase	Converts coA to Acetyl-CoA. Important for cell growth on acetate	1	1.33
	Acetate kinase	Acetate utilization	1	0.1
	Sulfite reductase	Convert sulfite to sulfide for energy production	1	1.37
	Methyltransferase	Reduce methanol to methane	2	0.64
	Others	Electron transport	10	Various
Cellular metabolism			*30*	
	Deoxyribonuclease	DNA degradation	1	1.77
	Proteasome subunit alpha	Protein degradation	2	0.73
	Pyridoxamine 5-phosphate oxidase	de novo synthesis of pyridoxine (vitamin B6) and pyridoxal phosphate.	1	0.71
	Effector protein	Elongation factor in protein biosynthesis	1	0.54
	Aspartate carbamoyltransferase	Nucleotide transport and metabolism	1	0.27
	NADP-dependent alcohol dehydrogenase	Catalyze the reversible oxidation of secondary and cyclic alcohols to the corresponding ketones	1	0.25
	Others	Various	23	Various
Oxidative stress response			*6*	
	Thioredoxin	Reduce hydrogen peroxide and certain radicals	1	2.27
	Superoxide dismutase	Reduce peroxide	2	0.19
	Universal stress protein	Resist stress	2	0.29
	Heat shock protein	Resist heat shock	1	0.26
Substrate transport			*5*	
	V-type ATP synthase subunit B	Use ATP hydrolysis to drive the transport of protons across a membrane	1	1.33
	Chain A, Crystal Structure Of The C-Terminal Duf1608 Domain	Part of a self-assembling proteinaceous surface (S-) layer	1	0.94
	ABC-type Fe3+-hydroxamate transport system, periplasmic component	Vitamin B12 bining protein for ABC transport	1	0.17
	ABC transporter substrate-binding protein	ATP-binding cassette transporters	1	0.1
	Basic membrane protein	Cell surface binding protein	1	0.11
Hypothetical proteins			*18*	
	Hypothetical protein	Unknown	1	6.27
	Hypothetical protein	Unknown	1	1.14
	Hypothetical protein	Unknown	1	1.2
	Hypothetical protein	Unknown	1	1.1
	Others	Unknown	14	Various

Task 4: Identification of enzymes secreted by archaea

Category	Protein name	Function	# of protein family	Abundance
Related to methane production			***40***	
	Methyl-coenzyme M reductase (Mcr)	Catalyze methane formation for all methanogens	7	5.09
	Methanol-5-hydroxybenzimidazolylcobamide methyltransferase	Reduce methanol to methane	5	0.56
	Methylene tetrahydromethanopterin dehydrogenase (Mtd)	H_2 forming for CO_2 reduction	2	0.75
	Methylene tetrahydromethanopterin reducatase (Mer)	The fifth enzyme in CO_2 reduction	3	0.13
	Formyl-methanofuran dehydrogenase (Fmd)	The first enzyme in CO_2 reduction	2	0.09
	Formate dehydrogenase (Fdh)	Enable cells to utililze formate as electron donor	2	0.12
	Acetyl-CoA synthase	Allow growth on acetate as sole source of carbon and energy	3	0.18
	Disulfide reductase	Catalyze the final step of methane production	1	0.06
	Phosphotransacetylase	Converts coA to Acetyl-CoA. Important for cell growth on acetate	1	1.33
	Acetate kinase	Acetate utilization	1	0.1
	Sulfite reductase	Convert sulfite to sulfide for energy production	1	1.37
	Methyltransferase	Reduce methanol to methane	2	0.64
	Others	Electron transport	10	Various
Cellular metabolism			***30***	
	Deoxyribonuclease	DNA degradation	1	1.77
	Proteasome subunit alpha	Protein degradation	2	0.73
	Pyridoxamine 5-phosphate oxidase	de novo synthesis of pyridoxine (vitamin B6) and pyridoxal phosphate.	1	0.71
	Effector protein	Elongation factor in protein biosynthesis	1	0.54
	Aspartate carbamoyltransferase	Nucleotide transport and metabolism	1	0.27
	NADP-dependent alcohol dehydrogenase	Catalyze the reversible oxidation of secondary and cyclic alcohols to the corresponding ketones	1	0.25
	Others	Various	23	Various

Task 4: Identification of enzymes secreted by archaea-con.

Category	Protein name	Function	# of protein family	Abundance
Oxidative stress response			***6***	
	Thioredoxin	Reduce hydrogen peroxide and certain radicals	1	2.27
	Superoxide dismutase	Reduce peroxide	2	0.19
	Universal stress protein	Resist stress	2	0.29
	Heat shock protein	Resist heat shock	1	0.26
Substrate transport			***5***	
	V-type ATP synthase subunit B	Use ATP hydrolysis to drive the transport of protons across a membrane	1	1.33
	Chain A, Crystal Structure Of The C-Terminal Duf1608 Domain	Part of a self-assembling proteinaceous surface (S-) layer	1	0.94
	ABC-type Fe3+-hydroxamate transport system, periplasmic component	Vitamin B12 bining protein for ABC transport	1	0.17
	ABC transporter substrate-binding protein	ATP-binding cassette transporters	1	0.1
	Basic membrane protein	Cell surface binding protein	1	0.11
Hypothetical proteins			***18***	
	Hypothetical protein	Unknown	1	6.27
	Hypothetical protein	Unknown	1	1.14
	Hypothetical protein	Unknown	1	1.2
	Hypothetical protein	Unknown	1	1.1
	Others	Unknown	14	Various



Sakai S, Takaki Y, Shimamura S, Sekine M, et al. (2011) Genome Sequence of a Mesophilic Hydrogenotrophic Methanogen Methanocella paludicola, the First Cultivated Representative of the Order Methanocellales. PLoS ONE 6(7): e22898. doi:10.1371/journal.pone.0022898.

Results

- Major compounds from coal depolymerization may be: sugars, amino acids, starch and collagen like chemicals, acetate, formate, C-4 carboxylates, etc.
- Bacterial species produced a significant amount of membrane proteins for substrate binding and transport, flagella proteins for cell movement and oxidoreductases for fighting against oxidative stress due to air exposure.
- For archaea strains, 41% of proteins were related to methane production
- We have identified major enzymes involved in methane production pathway, such as: Methyl-coenzyme M reductase (Mcr); Methanol-5-hydroxybenzimidazolylcobamide methyltransferase; Methylene tetrahydromethanopterin dehydrogenase (Mtd); Formyl-MFR dehydrogenase (Fmd); Formate dehydrogenase (Fd); Acetate kinase; Methyltransferase, etc.

Estimation of Surface Areas of Coal Residue

- Change in surface areas are estimated by change in the Langmuir Volume.
  - CH_4 sorption surface area:
    - ✓ Day 30 area = 1.5957 × Baseline area
    - ✓ Day 60 area = 4.45 × Baseline area
  - CO_2 sorption surface area:
    - ✓ Day 30 area = 1.4143 × Baseline area
    - ✓ Day 60 area = 2.21 × Baseline area

Task 1.0: Project management

Personnel involved

- Dr. Yanna Liang (PI)
- Dr. Satya Harpalani (Co-PI)
- Dr. Stephen Park (Postdoc, starting on Dec. 1st, 2014)
- Ji Zhang (Graduate student)
- Rohit Pandey (Graduate student)
- Zeying Zhu (Undergraduate student)

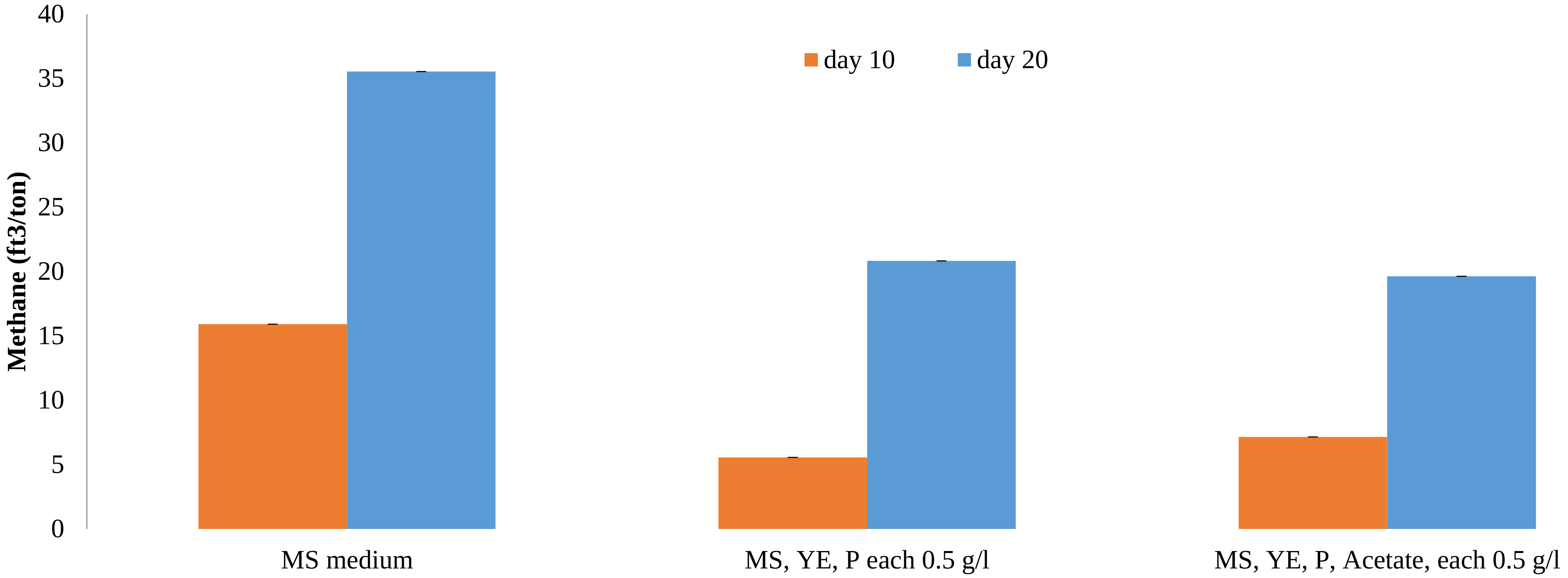
Task 2.0, *Subtask 2.1: Identify a simple medium recipe*

Cost of one liter medium

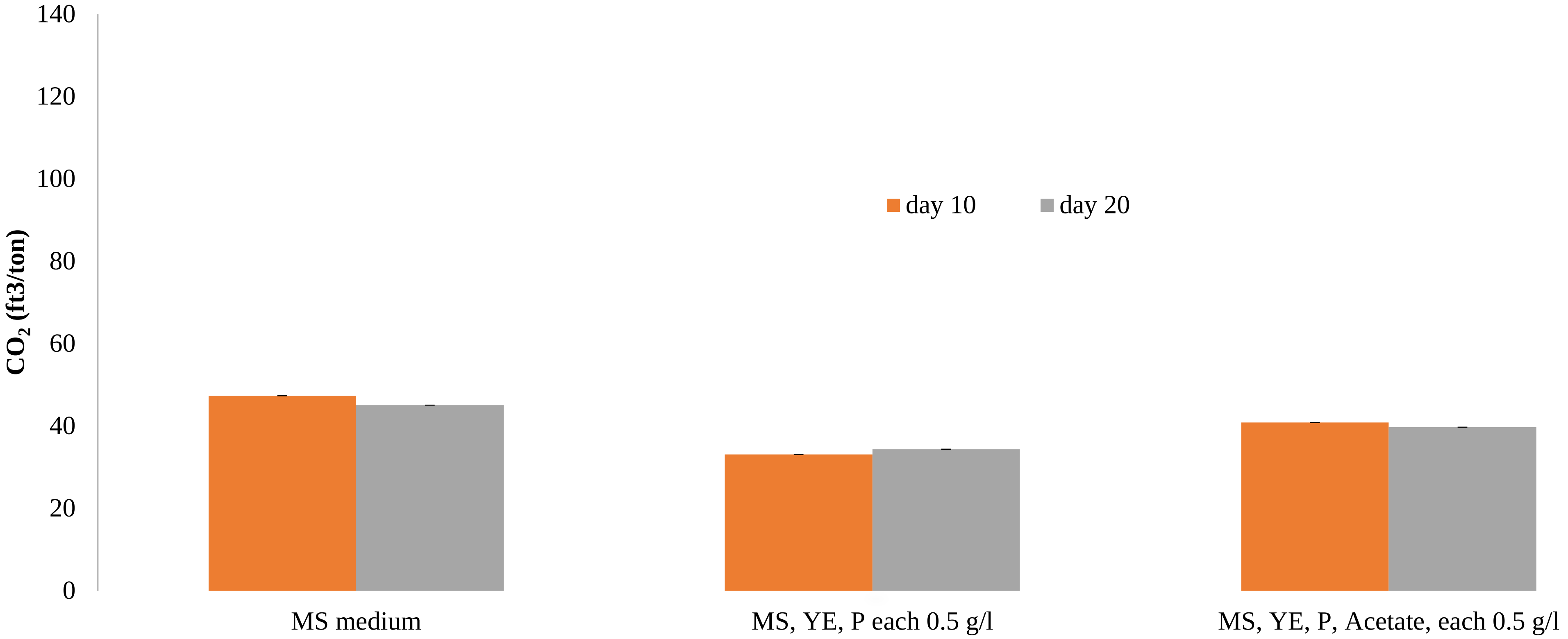
Minerals	**ACS certified Fisher price ($)**	**$ per liter**
$NaHCO_3$	694.43/300lb	0.0428
Yeast extract	382.85/5kg	0.1531
Trypticase peptones	2303.27/25lb	0.4059
Mercaptoethanesulfonic acid	144.97/25g	0.0029
$Na_2S.9H_2O$	260.27/2.5kg	0.0260
NH_4CL	311.64/10kg	0.0312
$K_2HPO_4.3H_2O$	1003.2/10kg	0.0401
$MgCl_2.6H_2O$	786.45/12kg	0.0655
$CaCl_2.2H_2O$	146.78/2.5kg	0.0235
Resazurin	156.8/25g	0.0063
		0.7973

Trace Mineral Solution	**ACS certified Fisher price ($)**	**$ per liter**
$NaEDTA.2H_2O$	1091.4/10kg	0.0005457
$CoCl_2.6H_2O$	290.24/500g	0.00087072
$MnCl_2.4H_2O$	238.34/2.5kg	9.5336E-05
$FeSO_{4.}7H_2O$	694.45/10kg	6.9445E-05
$ZnCl_2$	1679.9/50kg	3.3598E-05
$AlCl_3.6H_2O$	197.21/2.5kg	3.1554E-05
$Na_2WO_4.2H_2O$	225.99/500g	0.00013559
CuCl	171.12/2.5kg	1.369E-05
$Ni_2SO_4.6H_2O$	710.09/5 kg	2.8404E-05
H_2SeO_3	192.9/250g	0.00007716
H_3BO_3	543.56/10kg	5.4356E-06
$Na_2MoO_4.2H_2O$	478.65/2.5kg	1.9146E-05
		0.0019

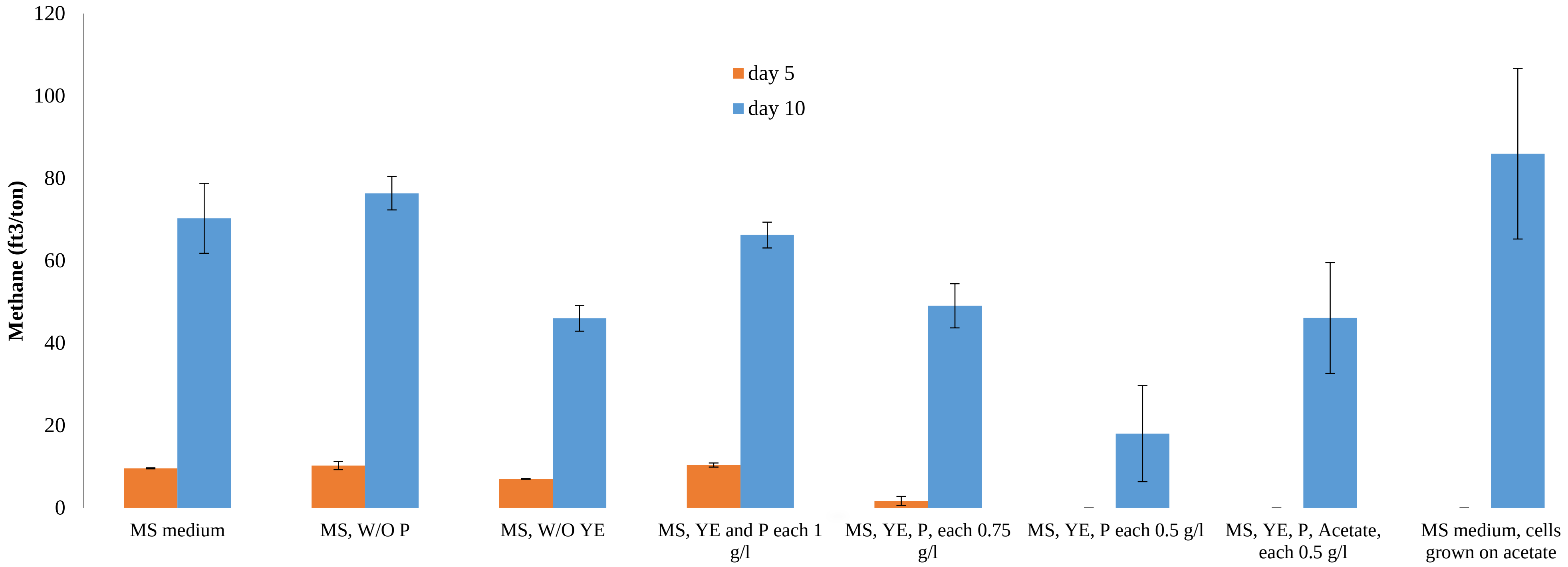
First exp.: Methane production. Microbial consortium after eight transfers



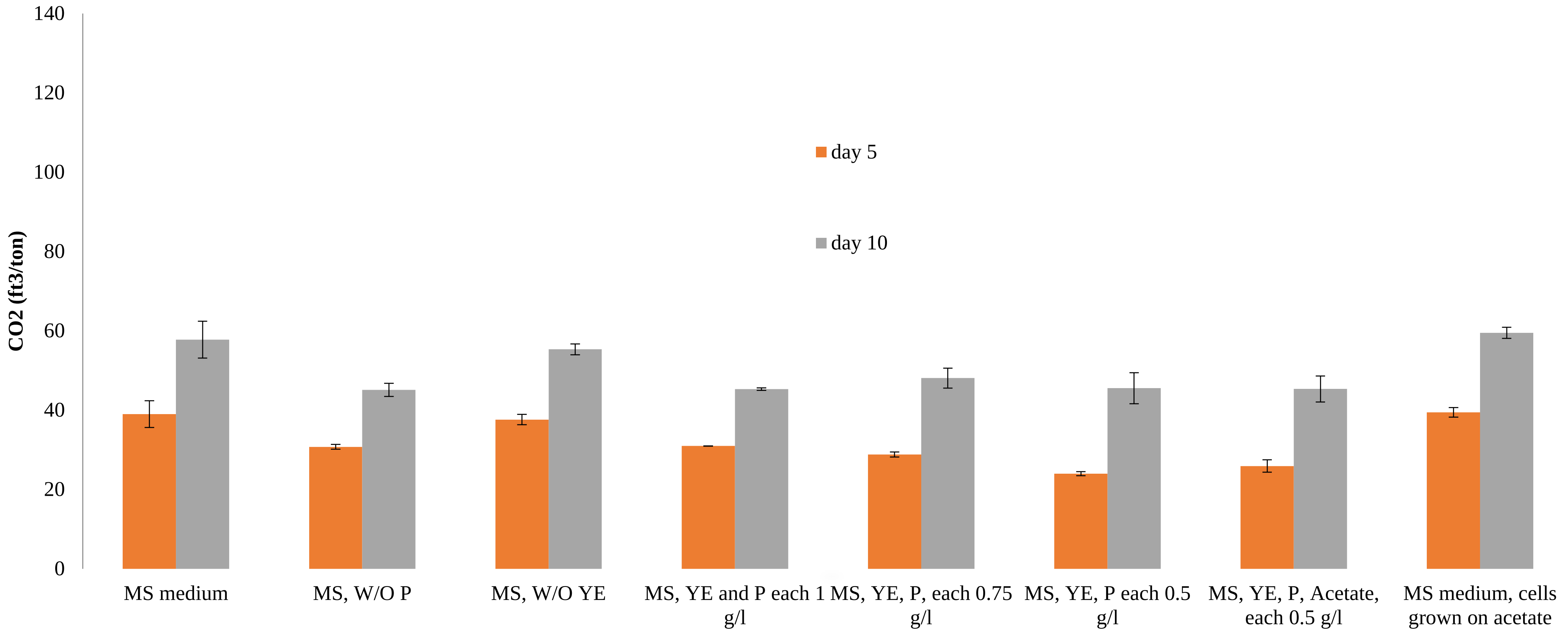
CO_2 release, Microbial consortium after eight transfers



Exp. 2: Methane production, newly adapted cells



CO2 release, newly adapted cells



Evaluate substitutes for yeast extract and peptone

No	Medium	Coal (g)	Cells (ml)	Medium + broth or liquor (ml)
1	MS medium	9	4.5	40.5
2	MS medium	9	4.5	40.5
3	MS medium, without TE, YE, Peptone, but with 50% of trypticase soy broth	0	4.5	40.5
4	MS medium, without TE, YE, Peptone, but with 50% of trypticase soy broth	0	4.5	40.5
5	MS medium, without TE, YE, Peptone, but with 50% of corn steep liquor	0	4.5	40.5
6	MS medium, without TE, YE, Peptone, but with 50% of corn steep liquor	0	4.5	40.5
7	MS medium, without TE, YE, Peptone, but with 10% of trypticase soy broth	9	4.5	40.5
8	MS medium, without TE, YE, Peptone, but with 10% of trypticase soy broth	9	4.5	40.5
9	MS medium, without TE, YE, Peptone, but with 30% of trypticase soy broth	9	4.5	40.5
10	MS medium, without TE, YE, Peptone, but with 30% of trypticase soy broth	9	4.5	40.5
11	MS medium, without TE, YE, Peptone, but with 50% of trypticase soy broth	9	4.5	40.5
12	MS medium, without TE, YE, Peptone, but with 50% of trypticase soy broth	9	4.5	40.5
13	MS medium, without TE, YE, Peptone, but with 10% of corn steep liquor	9	4.5	40.5
14	MS medium, without TE, YE, Peptone, but with 10% of corn steep liquor	9	4.5	40.5
15	MS medium, without TE, YE, Peptone, but with 30% of corn steep liquor	9	4.5	40.5
16	MS medium, without TE, YE, Peptone, but with 30% of corn steep liquor	9	4.5	40.5
17	MS medium, without TE, YE, Peptone, but with 50% of corn steep liquor	9	4.5	40.5
18	MS medium, without TE, YE, Peptone, but with 50% of corn steep liquor	9	4.5	40.5

Task 1.0, Subtask 1.2: *Characterize the coal samples*

- Elemental analysis

Sample name	Weight (mg)	Nitrogen %	Carbon %	Hydrogen %	Sulphur %	Oxygen %
Baseline Rep 1	2.033	1.399	70.319	5.234	0.646	15.269
Baseline Rep 2	3.501	1.381	69.816	5.190	0.609	15.408
Average		1.399	70.319	5.234	0.646	15.408
STDEV		0.013	0.356	0.031	0.026	0.197

Moisture content: 11.54%
Ash content: 6.11%
Dry ash free: $CH_{0.074}O_{0.22}N_{0.02}S_{0.009}$

Heat content: 12529.88 Btu/lb

Project outcomes

- One presentation was given by Ji Zhang at Mid-American Environmental Engineering Conference at Rolla, MO on Nov. 15th, 2014.
- One manuscript titled: "*Characterizing microbial communities dedicated for converting coal to methane in situ and ex situ*" is ready for submission to International Journal of Coal Geology.

Summary

- Subtask 1.1: Medium reduction is on-going.
- Subtask 1.2: Proximate analysis regarding fixed carbon is on-going.
- Evaluate methods for long-term storage of the adapted microbial consortium.
- Task 7.0: TEA analysis of the baseline data through using Aspen plus is ongoing.

Questions, Comments, Suggestions