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Biological hydrogen production

- Hydrogen as an energy carrier
- Routes for hydrogen production
- Fermentation
  - Mesophilic vs. Thermophilic
- Examples of data

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Hydrogen as energy carrier

- Hydrogen
  - Clean renewable energy source
  - Highest energy density of any fuel (carbon free)
  - Contributes substantially to the reduction of greenhouse gas emissions.
  - H_2 from renewable sources might be considered as the ultimate clean and climate neutral energy system

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Comparison of energy and emissions of combustable fuels

Fuel type	Energy/unit (MJ/kg)	Energy/vol (MJ/l)	Kg of C release/kg fuel used
Hydrogen gas	120	2	0
Hydrogen liquid	120	8,5	0
Coal	15-30		0.6
Natural gas	33-50	9	0.46
Petrol	40-43	31,5	0.86
Oil	42-45	38	0.84
Diesel	43	35	0.9
Bio-Diesel	37	33	0.5
Ethanol	21	23	0.5
Charcoal	30		0.5
Agric. Residues	10-17		0.5
Wood	15		0.5

From Vijayaraghavan & Soom, 2005 (Int. J. Hydrogen Energy)

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Unit cost of energy obtained by different processes

Type of Energy	Conversion effciency (%)	Unit cost (US$)
H2 (photobiological)	10	**10**
H2 (fermentation)	10	**40**
H2 (coal/biomass)		**4**
H2 (electrolysis)		10
H2 (thermal decompostion		13
H2 (photochemical)		21
Ethanol (fermentation)	15-30	32
Gasoline		6

From: Das & Veziroglu, 2001 (Int. J. Hydrogen Energy)

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Comparison of different biological hydrogen production processes

Organisms	Example	Raw material	DT (h)	Max rate (mmol H2/h)	Max rate (mmol H2/g dry cell/h)	Major products
Photo						
Double PS	Oscillatoria	Sp. Media		0.4	0.3	H2/CO2/O2
	Anabaena	Various	25	1.2	1.3	H2/O2
Single PS	Rhodopseudomonas	Various			0.3-2.0	H2/CO2/O2 (VFA)
	Rhodobacter	Various			0.05-5.9	H2/CO2/O2 (VFA)
Fermentative	C. butyricum	Glucose			7.3	H2/CO2/VFA
	Citrobacter	Cellulose		11	9.5	H2/CO2/VFA
	E. aerogenes	Sugar cane	0.25	11.36	17	H2/CO2/VFA
	E. cloacae	Sucrose	0.32	37.03	29.63	H2/CO2/VFA
	C. saccharolyticus	Sucrose		8.4		H2/CO2/VFA
	T. elfi	Glucose		2.7-4.5		H2/CO2/VFA

From: Levin et al. 2004; Das & Veziroglu, 2001

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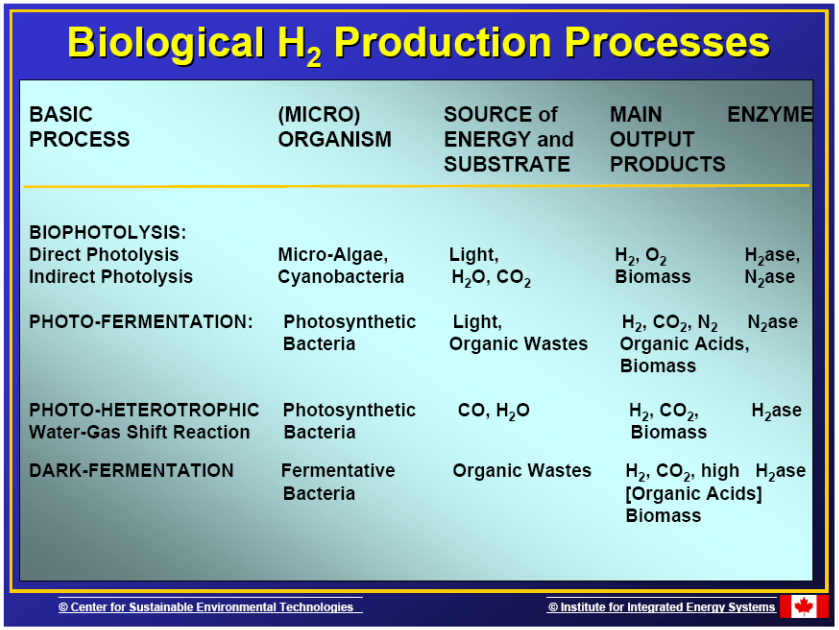
Biological hydrogen production - routes

- Hydrogen production by
  - Direct biophotolysis
  - Indirect biophotolysis
  - Photo-fermentation
  - Dark-fermentation



Biological H_2 Production Processes

BASIC PROCESS	(MICRO) ORGANISM	SOURCE of ENERGY and SUBSTRATE	MAIN OUTPUT PRODUCTS	ENZYME
BIOPHOTOLYSIS: Direct Photolysis Indirect Photolysis	Micro-Algae, Cyanobacteria	Light, H_2O, CO_2	H_2, O_2 Biomass	H_2ase, N_2ase
PHOTO-FERMENTATION:	Photosynthetic Bacteria	Light, Organic Wastes	H_2, CO_2, N_2 Organic Acids, Biomass	N_2ase
PHOTO-HETEROTROPHIC Water-Gas Shift Reaction	Photosynthetic Bacteria	CO, H_2O	H_2, CO_2, Biomass	H_2ase
DARK-FERMENTATION	Fermentative Bacteria	Organic Wastes	H_2, CO_2, high [Organic Acids] Biomass	H_2ase

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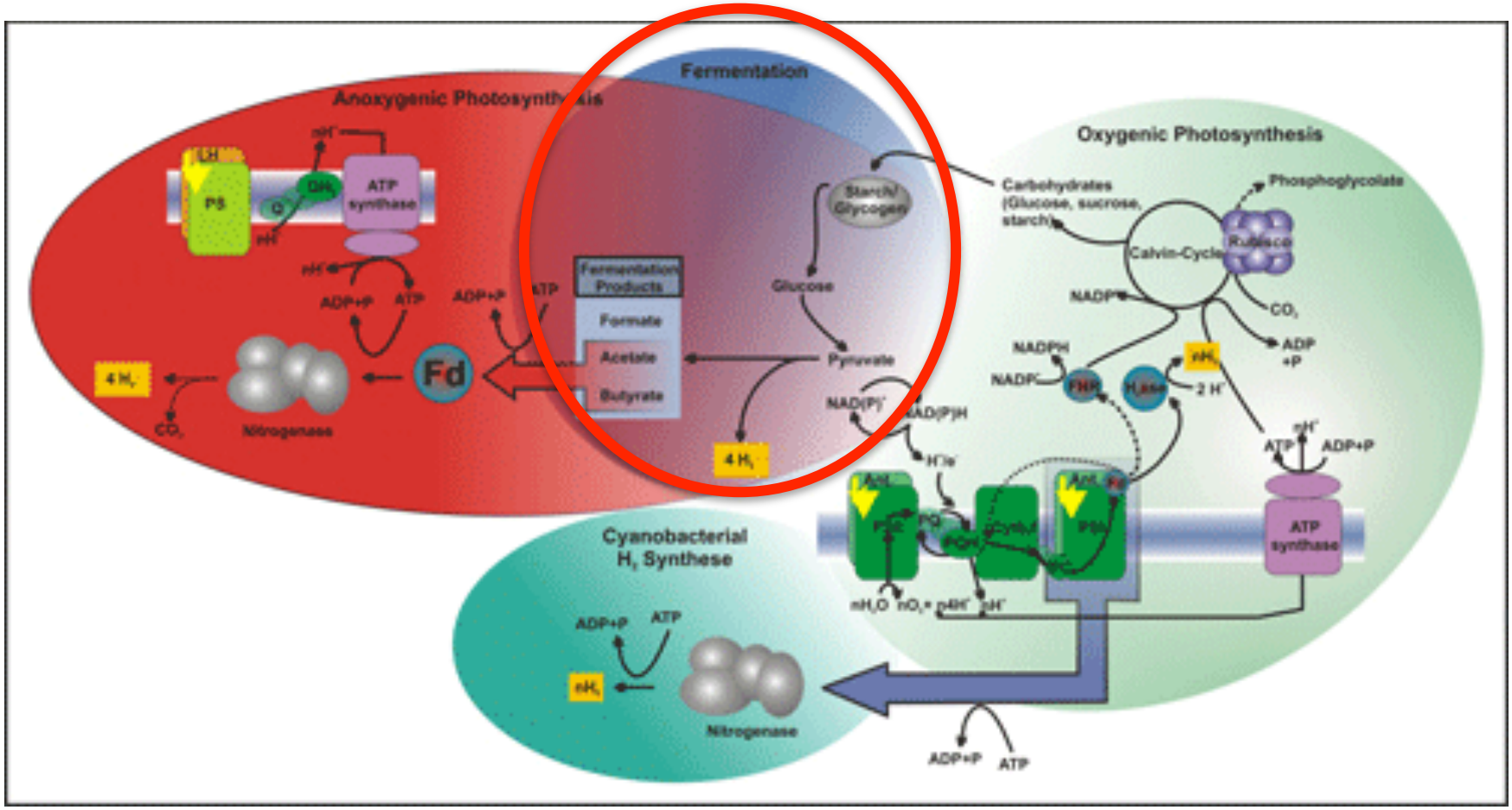
Biological hydrogen production - routes



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Dark fermentation

- Hydrogen can be produced by anaerobic bacteria, grown in the dark on carbohydrate-rich substrates
  - Mesophilic (25–40°C)
  - Thermophilic (40–65°C)
  - Extreme thermophilic (65–80°C)
  - Hyperthermophilic (>80°C)

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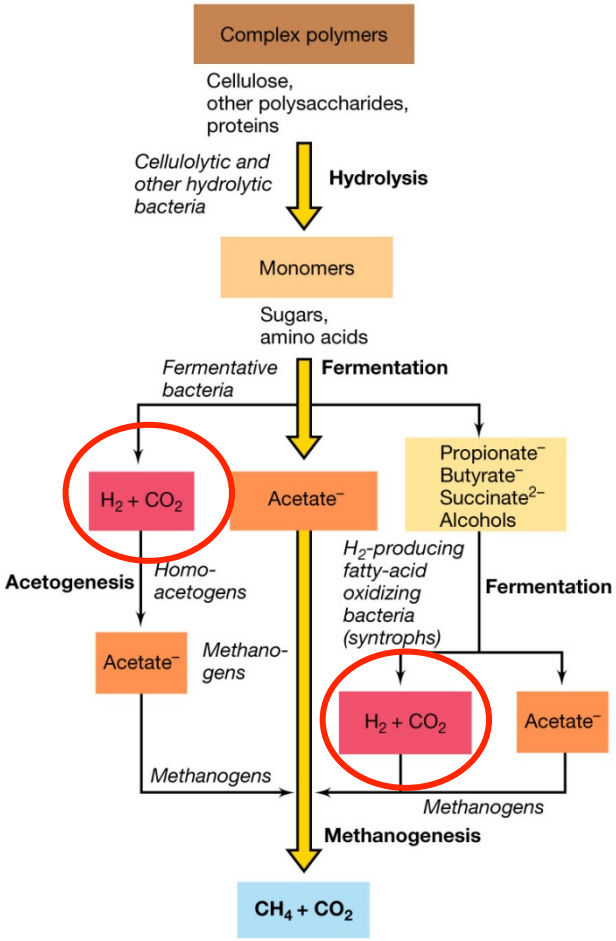
Anaerobic digestion



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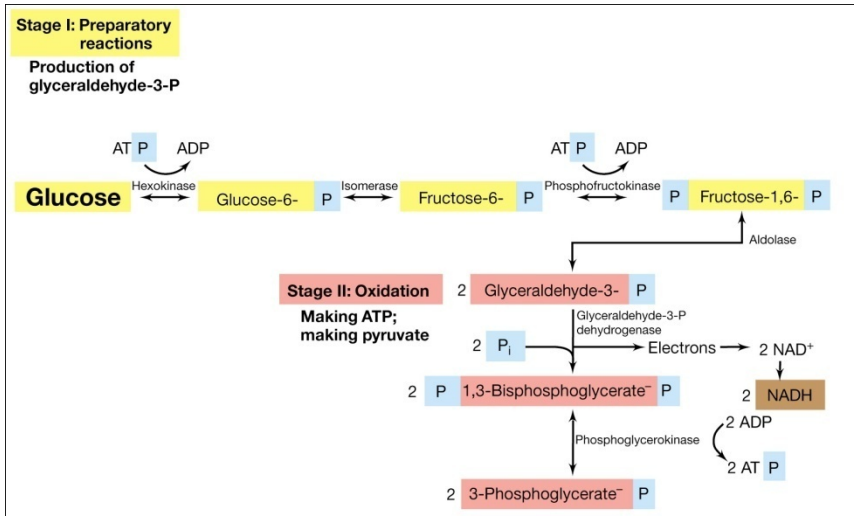
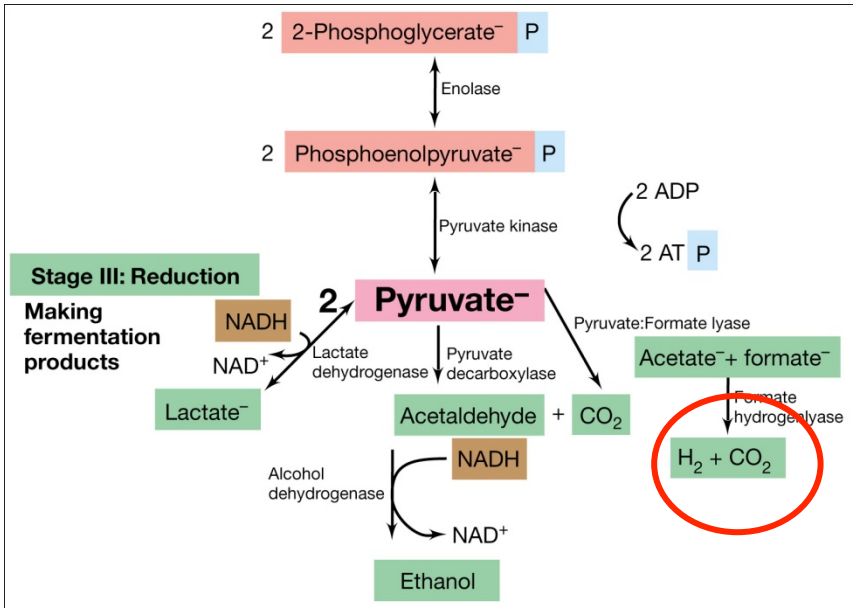
Glycolysis





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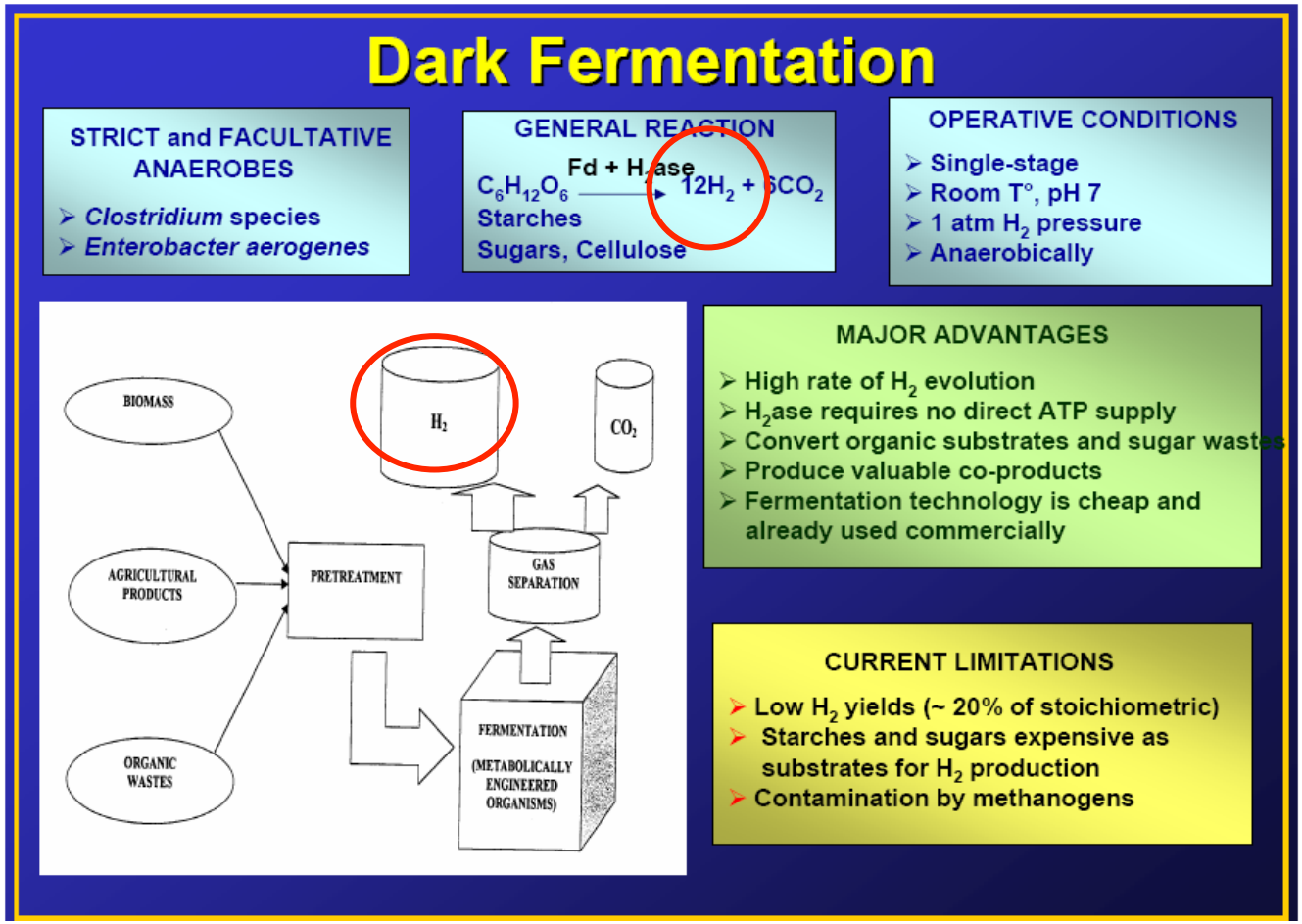
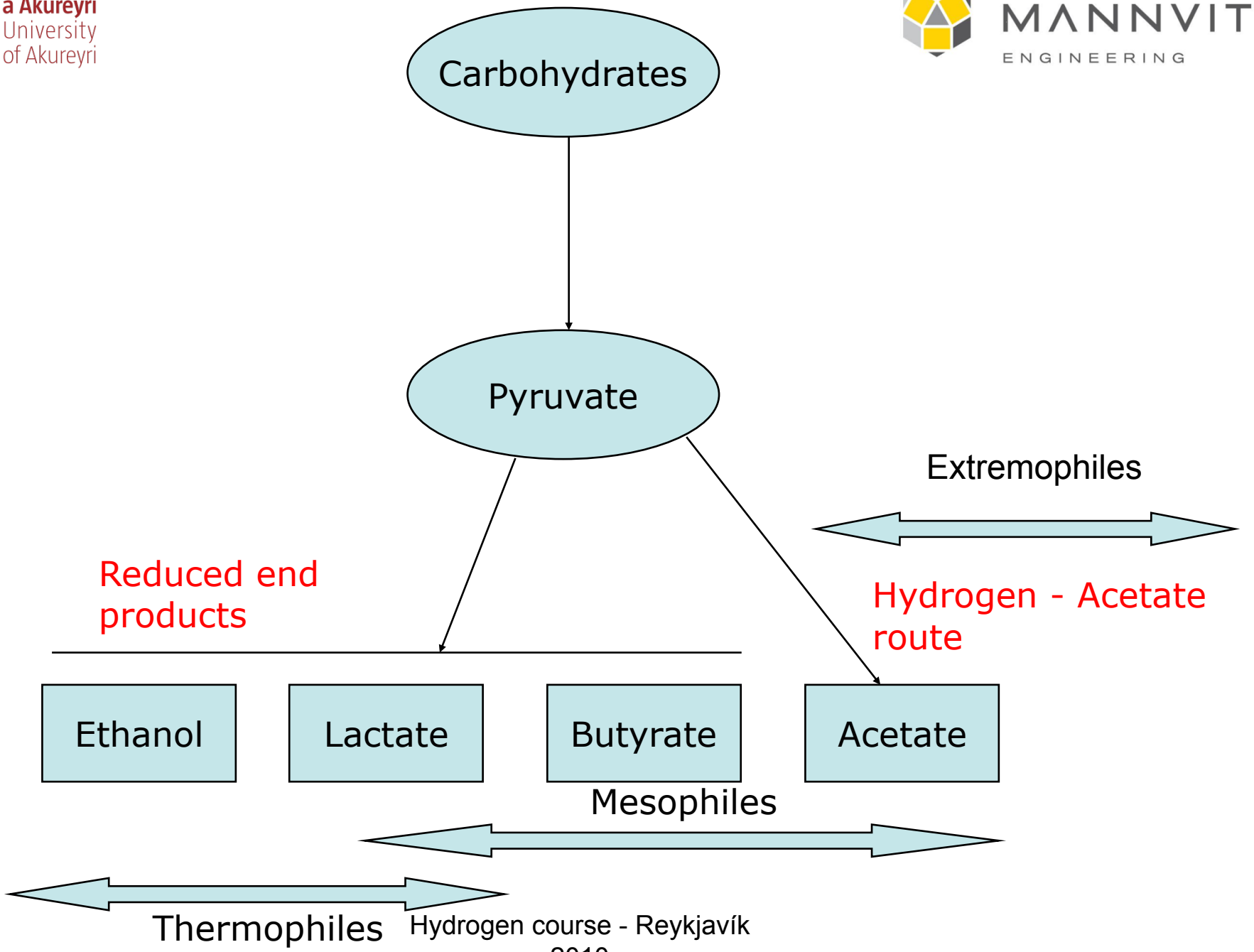
Fermentation




Carbohydrates
Pyruvate
Extremophiles
Reduced end products
Hydrogen - Acetate route
Ethanol
Lactate
Butyrate
Acetate
Mesophiles
Thermophiles

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Hydrogen production from carbohydrates

- Carbohydrates yield different amounts of H_2 depending on the fermentation pathway and end products
  - $C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ H_2$
  - $C_6H_{12}O_6 + 4\ H_2O \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2\ H_2$
- Acetate → highest hydrogen yields
- In practice – mixture of Ac/But and other products → lower yields

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Hydrogen production from carbohydrates

- Processes important for H2 production
  - pH
  - HRT (in continuous cultures)
  - pH_2
  - Initial substrate concentrations
  - → Fermentation end products depend on the environmental conditions

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Hydrogen production from carbohydrates

- Reduced fermentation end products are produced when H_2 accumulates
  - Etanol, Lactate, Butanol, Alanine
- To maximize the yield of H_2 the metabolism must be directed away from alcohols and reduced acids

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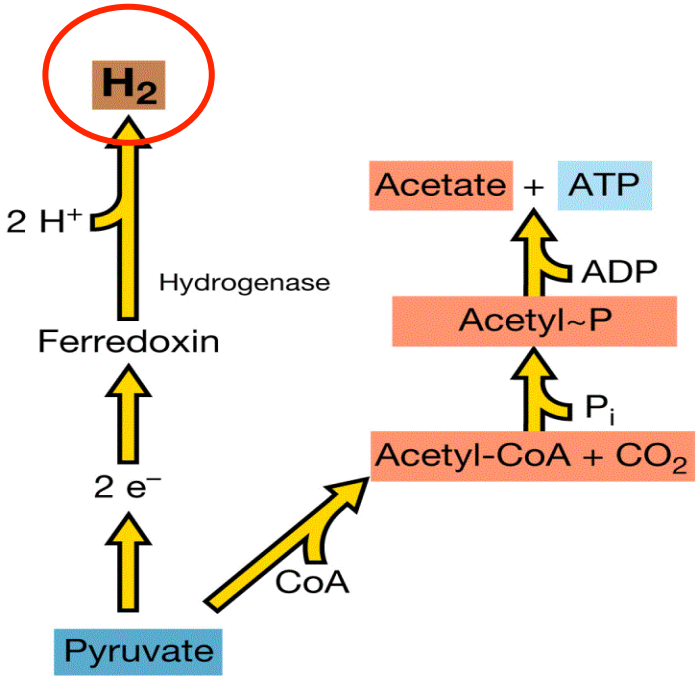
Fermentation

- The majority of microbial H_2 production is driven by the anaerobic metabolism of pyruvate formed during the catabolism of various substrates.
- The breakdown of pyruvate is catalyzed by one of the two enzyme systems:
  - Pyruvate: formate lyase (PFL)
    - (Pyruvate + CoA) → acetyl-CoA + formate
  - Pyruvate: ferredoxin (avodoxin oxido reductase (PFOR)
    - Pyruvate + CoA + 2 Fd (ox) → acetyl-CoA + CO2 + 2 Fd (red)



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Fermentation

- Pyruvate → acetyl-CoA → ATP + formate/reduced ferredoxin → H_2
- The overall yields are relatively low,
  - 1-2 H_2 per molecule of pyruvate.
- → fermentations have been optimized by evolution to produce biomass and not H_2.
- Thus, a portion of the substrate (pyruvate) is used in both cases to produce ATP, giving a product (acetate) that is excreted.

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Fermentation - thermodynamics

- The major issue is the feasibility of a dark fermentative reaction yielding close to the 12 mol H_2 stored in each molecule of glucose metabolized.
- From a thermodynamic perspective, the most favourable products from the breakdown of 1 mol of glucose gives rises to 2 mol of acetate and 4 mol of H_2.
  - In reality: Maximum 3.3 mol H_2

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pH_2

- pH_2 is extremely important for H_2 production
- H_2 synthesis pathways are sensitive to H_2 conc. and are subject to end product inhibition
- Previously: ↑ H_2 → ↑ pH_2 → slower down H_2 production
- Thermodynamics: ↑T°C → pH_2 has less effect
- Thus at higher temperatures oxidative reactions can occur to a more extent before reduced compound are produced AND inhibition starts at higher pH_2

Mesophilic hydrogen producing bacteria

- Strict anaerobes
  - *Clostridium*
  - *Citrobacter*
  - *Klebsiella*
  - *Enterobacter*

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Thermophilic hydrogen producing bacteria

- *Clostridium*
- *Caloramator*
- *Thermoanaerobacter*
- *Thermoanaerobacterium*
- *Caldicellulosiruptor*
- *Thermotoga*

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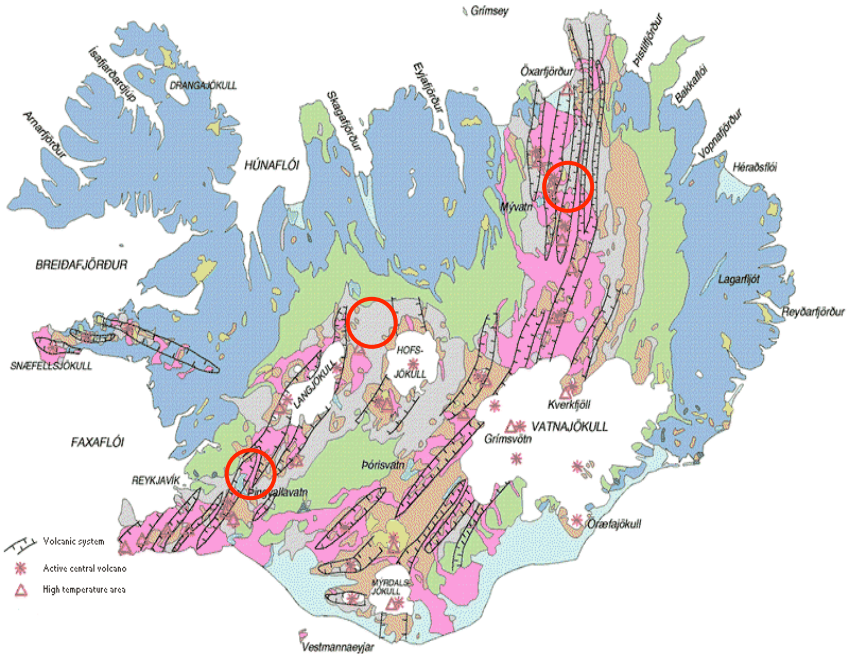
Results

- Samples were collected in four trips from three different geothermal areas in 2004, 2005, 2007 and 2009



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Results

- Isolations of hydrogen producing strains
- Basic physiological characteristics
- Substrate spectrum
- Influence of initial substrate concentrations
- Influence of pH_2
- Continuous culture
- Complex biomass studies

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Physiological data of sampling sites

- Örlygsson and Baldursson, 2007.
- - 4 strains isolated
- AK1 and AK14 (both *Clostridium*)
  - Isolated from Grensdalur (Hveragerdi)
- AK15 (Clostridium) and AK17 (*Thermoanerobacterium*)
  - Isolated from Hell (Víti)

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Sampling and environment

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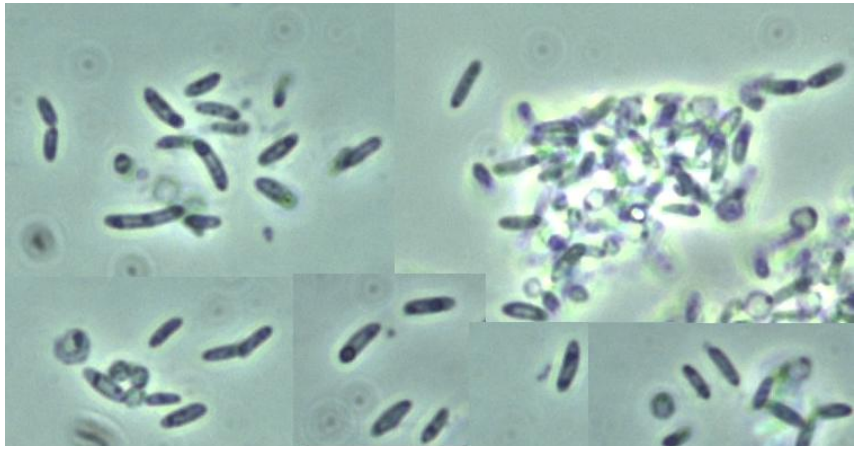
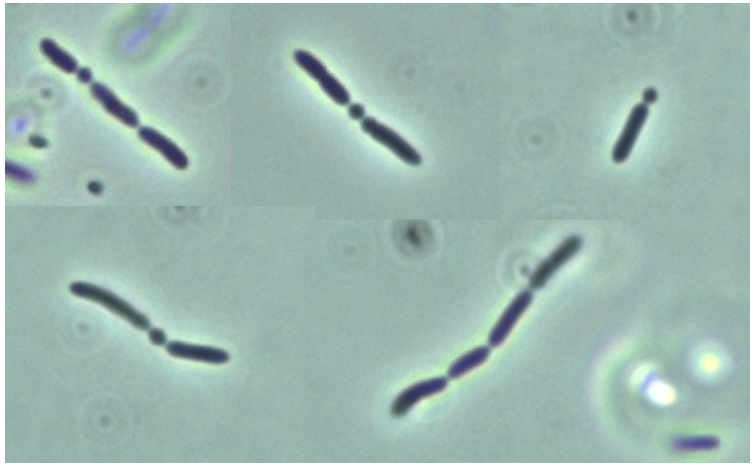
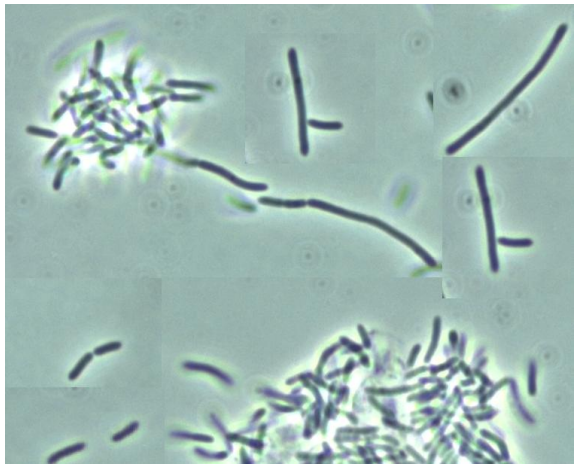
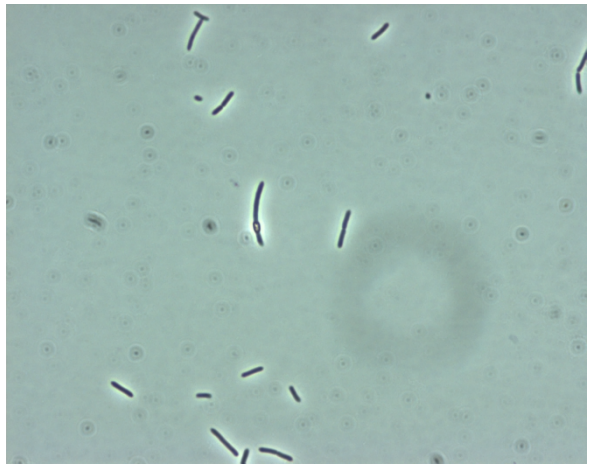
Light microscope









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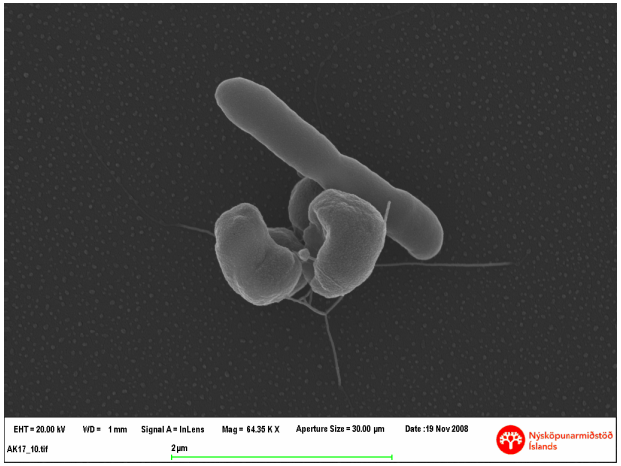
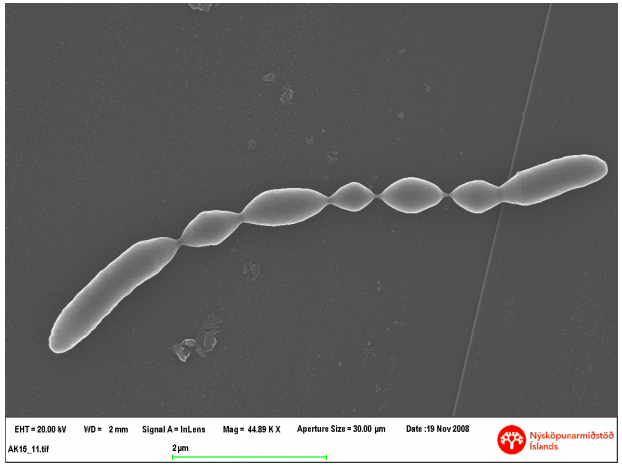
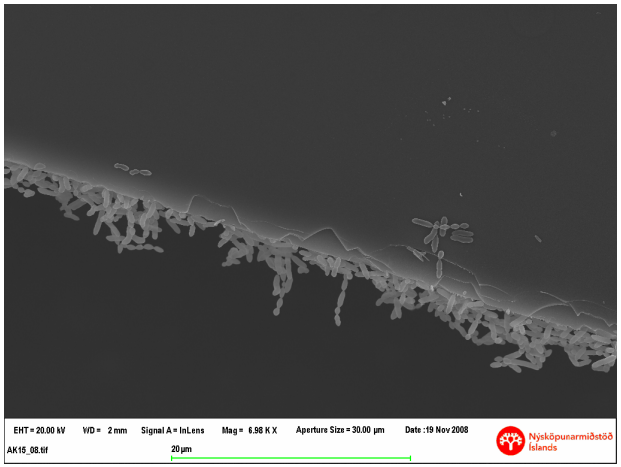
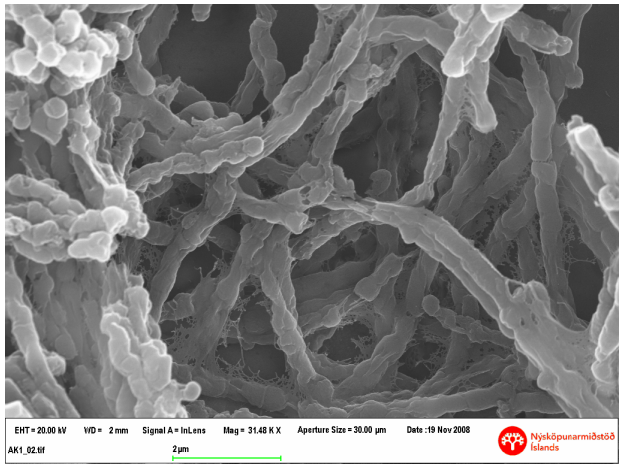
Electron microscopy









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Physiological data

	AK_1	AK_{14}	AK_{15}	AK_{17}
T_{opt}	45	45-50	55	58
T_{max}	55	55.68	80	72
pH_{opt}	7.0 - 8.0	7.0	7.0	4.5-6.5
μmax	0.16	0.44	0.25	0.4
Gen time	4.0	1.6	2.7	2.0

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	Strain			
Substrate	**AK_1**	**AK_{14}**	**AK_{15}**	**AK_{17}**
Arabinose	-	-	-	++
Fructose	+++	+++	+++	++
Galactose	++	+++	+	+++
Glucose	++	++++	+++	+++
Mannose	++	+++	+++	+++
Ribose	-	-	++	+++
Xylose	+	+++	++	+++
Lactose	++	-	-	++
Sucrose	++	++++	++	++++
Cellulose	-	-	-	(+)
Pectin	++	-	-	(+)
Xylan	-	-	++	-
Pyruvate	-	-	+	-
Serine	-	-	-	++
Threonine	-	-	-	++

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C-source	Strain AK_1	Strain AK_{14}	Strain AK_{15}	Strain AK_{17}
	mol/mol c-source			
Arabinose	-	-	0.69	0.33
Fructose	0.61	1.0	0.16	0.73
Galactose	0.7	1.07	0.63	0.74
Glucose	0.77	1	0.7	0.55
Mannose	0.77	0.81	-	1.22
Ribose	0.07	-	0.57	1.02
Xylose	0.71	1.14	-	0.69
Lactose	0.76	-	0.67	-
Sucrose	0.58	1.04	-	1.14
Cellulose	-	-	nd	nd
Pectin	nd	-	-	nd
Xylan	nd	-	0.12	-
Pyruvate	0.06	0.1	-	0.52
Serine	-	-	-	0.62
Threonine	-	-		0.52

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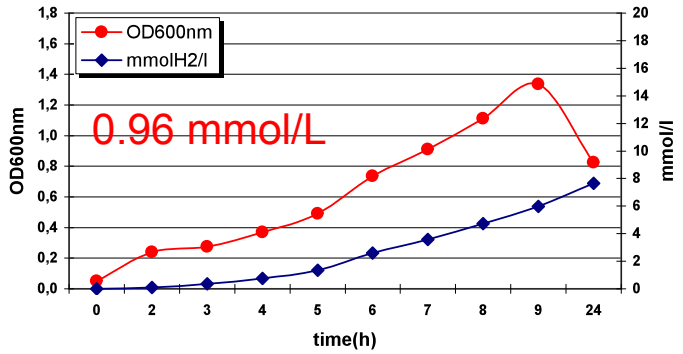
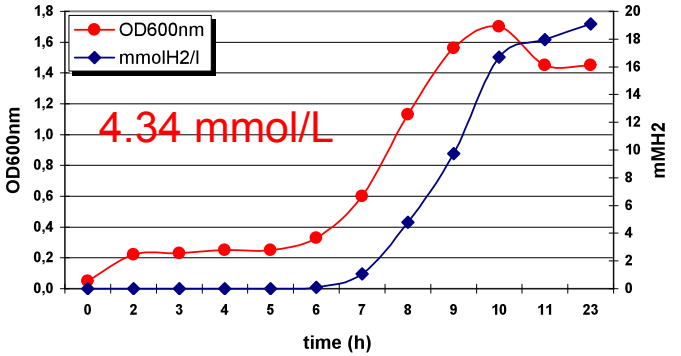
Hydrogen production rates

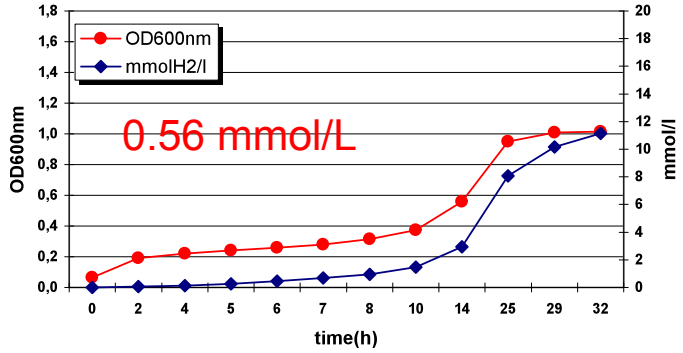
Strain AK_1



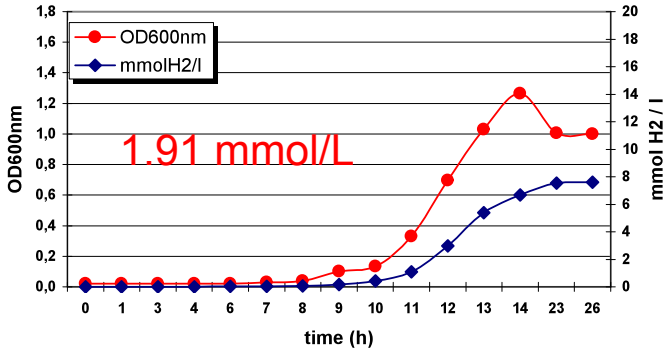
Strain AK_{14}



Strain AK_{15}



Strain AK_{17}



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Fermentation end products

Strain / mmol/l	Ethanol	Acetate	Butyrate	Lactate	Hydrogen	CO_2
AK_1	29.7	10.5	0.0	4.0	7.7	40.3
AK_{14}	0.0	8.3	12.0	3.3	19.1	32.3
AK_{15}	16.6	8.3	0.0	3.5	11.1	24.9
AK_{17}	31.2	11.4	0.0	0.0	7.6	42.6

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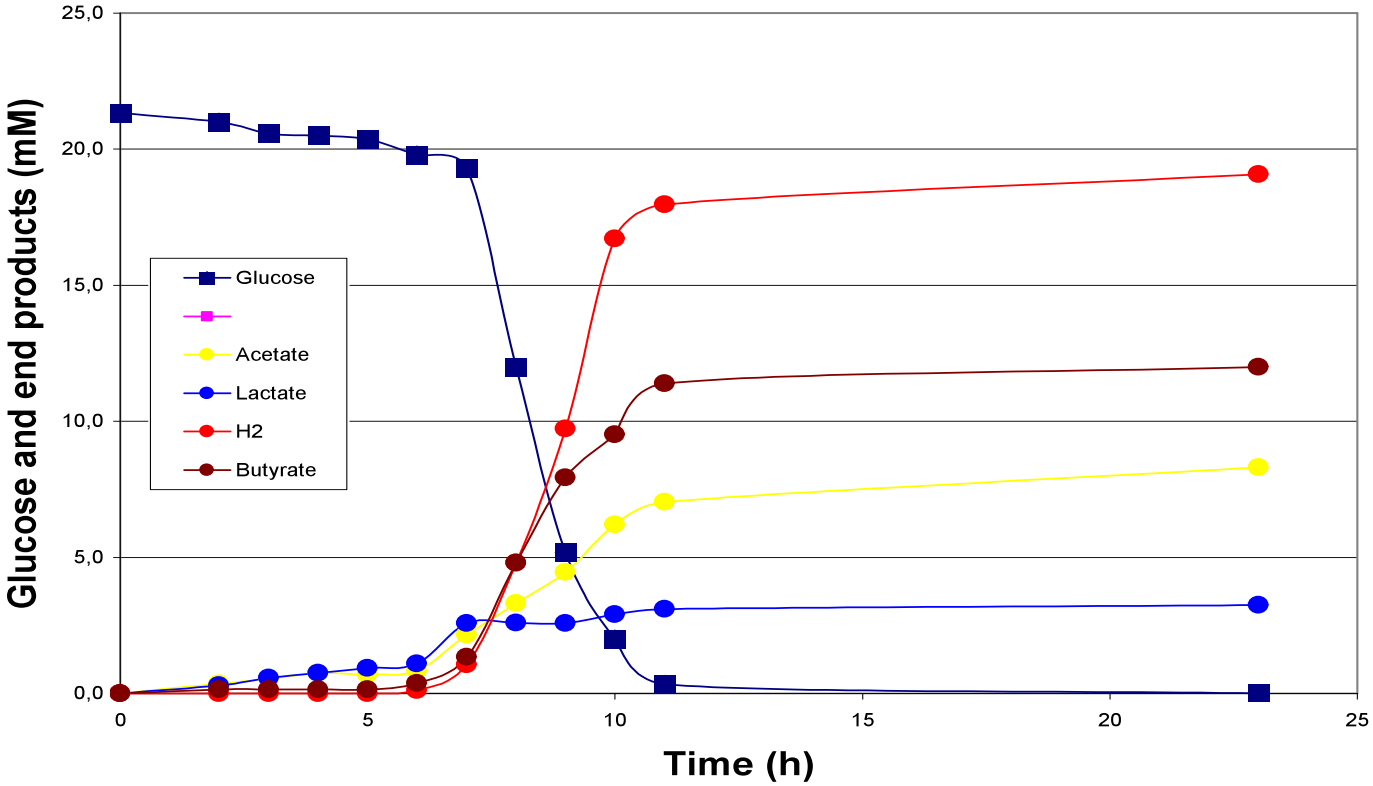
AK14



Hydrogen course - Reykjavík
2010

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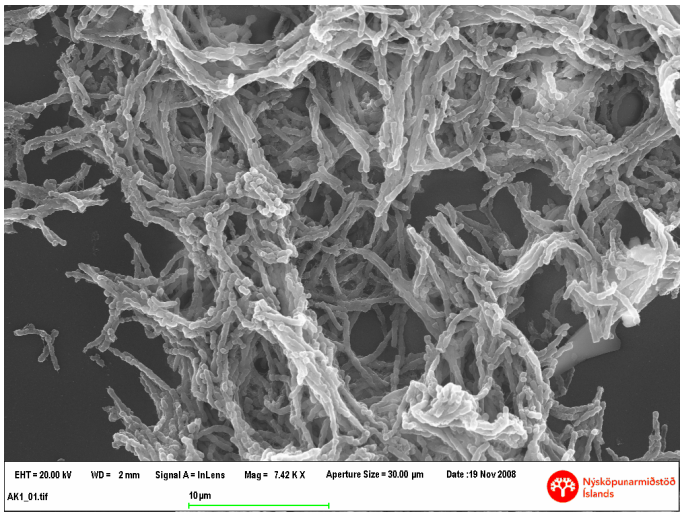
Continuous culture

- Ethanol and Hydrogen Production by Two Thermophilic, Anaerobic Bacteria Isolated From Icelandic Geothermal Areas
  - Biotechnology and Bioengineering
    - Two bacterial strains were isolated from two sediment samples collected in the Krafla area (Víti=Hell) in NE-Iceland
      - AK15: 60°C; pH = 8.6
      - AK17: 70°C; pH = 6.5



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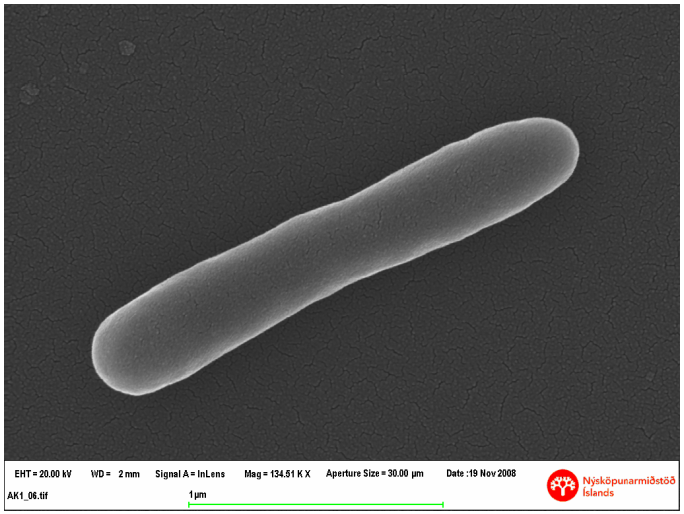
Batch fermentation patterns from glucose and xylose by the isolate AK17

- Glucose –
  - Hydrogen main fermentation product
    - 0.4-1.2 mol H_2/mol glucose
  - Ethanol
    - 1.2-1.6 mol EtOH/mol glucose
  - Acetate
    - 0.5 mol/mol glucose
- Xylose
  - Hydrogen
    - 0.9-1.0 mol H_2/mol xylose .
  - Acetate was the main soluble metabolite
  - Ethanol
    - 1.0-1.1mol/mol xylose
  - Acetate - less



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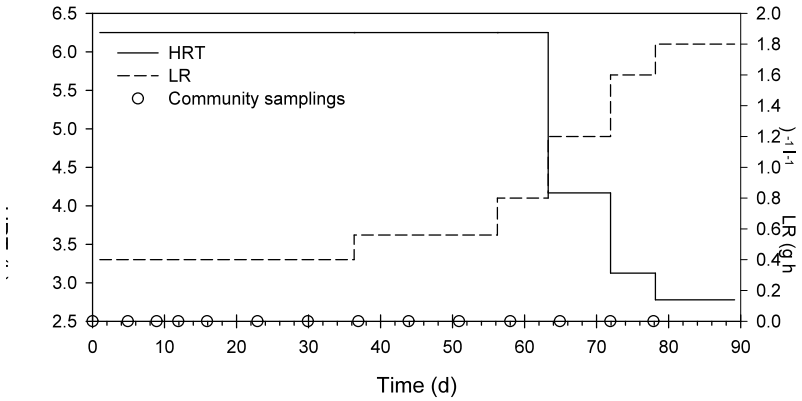

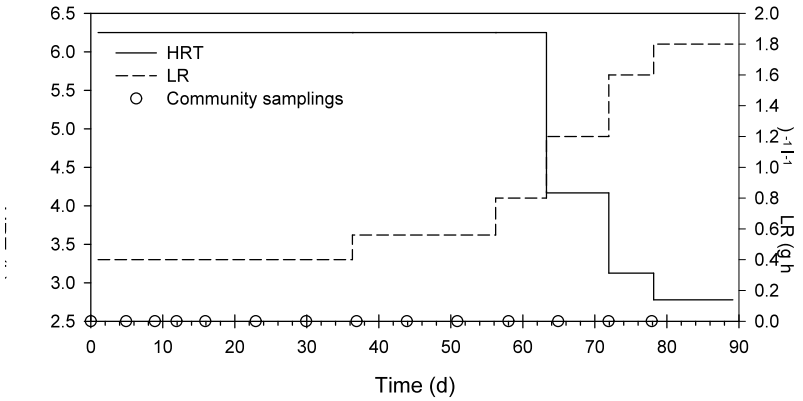

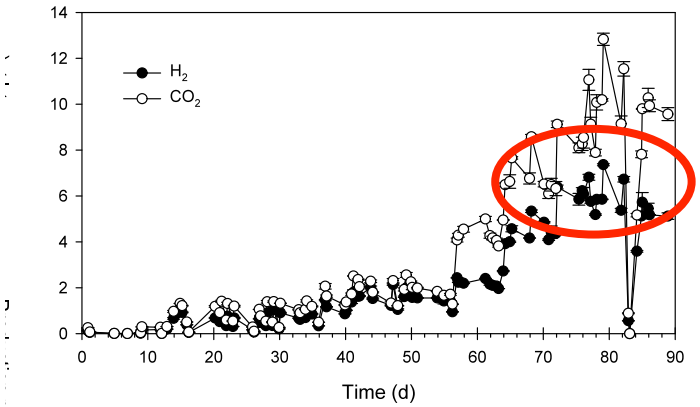

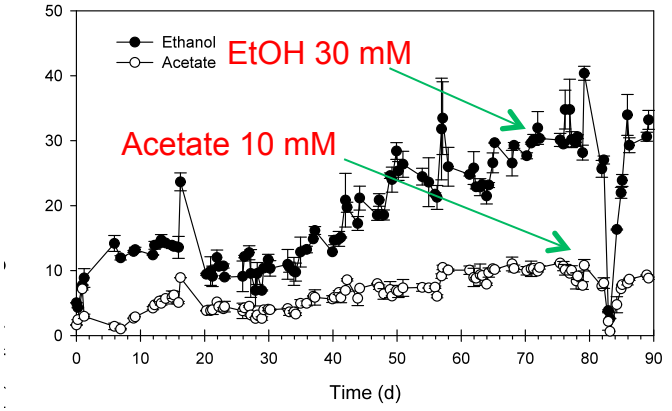

Hydrogen course - Reykjavík
2010

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Compared to literature

Table III. The comparison of hydrogen production performance of some continuous-flow bioreactors reported in the literature.

Substrate	Temperature (°C)	H_2 yield (mol-H_2/mol-hexose)	H_2 production rate (mmolH_2/L)	References
Glucose	60	0.80	6.1	This study
Glucose	60	1.11	43.8[a]	Oh et al. (2004)
Sugar factory wastewater	60	2.57	8.3[a]	Ueno et al. (1996)
Winery wastewater	55	2.14	6.6[a]	Yu et al. (2002b)
Glucose	70	2.47	2.1[a]	Kotsopoulos et al. (2006)
Glucose	74	0.42	1.4	Koskinen et al. (2008)
Cellulose powder	60	2.00	1.2[a]	Ueno et al. (2001)
Sucrose	40	1.59[a]	627[a]	Wu et al. (2006)
Glucose	37	1.71	311[a]	Zhang et al. (2008)
Fructose	35	0.56	33.0	Wu and Chang (2007)
Glucose	35	1.71[a]	29.6[a]	Lin and Chang (1999)
Glucose	30–34	0.86[a]	15.0[a]	Lin and Chang (2004)

[a]Calculated based on the information provided.

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Bioprospecting hydrogen producers

- Icelandic Agricultural Sciences, 2010
- Purpose – Isolate and characterize thermophilic saccharolytic bacteria
- New enrichment experiments
  - Hveragerdi (SW Iceland) and Krafla (NE Iceland)

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Methods...

- Geysers at 50 – 80°C and pH 3 – 7.
- Carbon sources: glucose, xylose, cellulose, pectin, xylan
- # samples = 47 x 6 → 282
- Glucose and xylose = 100 mM
- Polymers = 5 g/L
- Growth follwed by H_2 measurements
- Best growth → end point dilutions and agar plates
- → phylogenetic analysis (16S rRNA)

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Selection of strains

- Aim: Characterize all strains concerning end product formation
- The best strains (↑EtOH, ↑H_2) chosen
- Plus = growing on many types of sugars and polysaccharides
- Hydrogen producers: EtOH/Acetate = < 1
- Ethanol producers: EtOH/Acetate = > 3

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Enrichment cultures

Enrichment culture	T (°C) (site)	pH (site/isolation)	Enrichment culture	T (°C) (site)	pH (site/isolation)	Enrichment culture	T (°C) (site)	pH (site/isolation)
50°C-samples			52-07-P	50	5.4/6.0	66-07-G	62	7.4/7.0
1-07-Cpa-G	45	8.0/7.0	65-07-Xo	49	6.7/7.0	66-07-P	62	7.4/7.0
2-07-G	50	7.9/7.0	65-07-X	49	6.7/7.0	67-07-P	66	7.7/7.0
2-07-Cpa-G	50	7.9/7.0						
2-07-Cpa	50	7.9/7.0	**60°C samples**			**70°C samples**		
9-07-P	40	4.8/6.0	8-06-G*	58	6.2/6.0	2-06-G*	73	4.3/7.0
9-07-X	40	4.8/6.0	21-07-Xo	60	6.7/7.0	4-07-X	69	8.0/7.0
10-07-P	46	6.6/7.0	21-07-Cpo	60	6.7/7.0	8-07-Cpo	71	8.2/7.0
10-07-X	46	6.6/7.0	21-07-Cpa-G	60	6.7/7.0	8-07-Cpa	71	8.2/7.0
15-06-G*	49	5.8/7.0	24-07-X	60	7.7/7.0	20-07-G	69	7.5/7.0
15-07-Cpa-G	50	7.5/7.0	27-07-X	60	7.7/7.0	20-07-X	69	7.5/7.0
23-07-Cpa-G	57	7.7/7.0	29-07-G	60	9.6/7.0	20-07-Cpo	69	7.5/7.0
25-07-Cpa-G	50	7.4/7.0	29-07-Cpo	60	9.6/7.0	33-07-Xo	71	8.0/7.0
35-07-X	50	7.7/7.0	29-07-Cpa-G	60	9.6/7.0	55-07-Xo	73	5.1/6.0
35-07-Cpa-G	50	7.7/7.0	34-07-X	60	7.4/7.0			
44-07-G	56	5.5/6.0	54-07-Xo	66	5.3/6.0	**75°C samples**		
44-07-Xo	56	5.5/6.0	54-07-P	66	5.3/6.0	14-07-G	84	8.0/7.0
44-07-P	56	5.5/6.0	63-07-G	60	7.7/7.0	14-07-Xo	84	8.0/7.0
44-07-X	56	5.5/6.0	63-07-Cpa	60	7.7/7.0	14-07-P	84	8.0/7.0
47-07-Xo	53	6.2/6.0	64-07-G	59	7.0/7.0	14-07-X	84	8.0/7.0
47-07-P	53	6.2/6.0	64-07-P	59	7.0/7.0	32-07-G	78	4.9/6.0
52-07-Xo	50	5.4/6.0	64-07-X	59	7.0/7.0	39-06-G*	78	5.5/6.0

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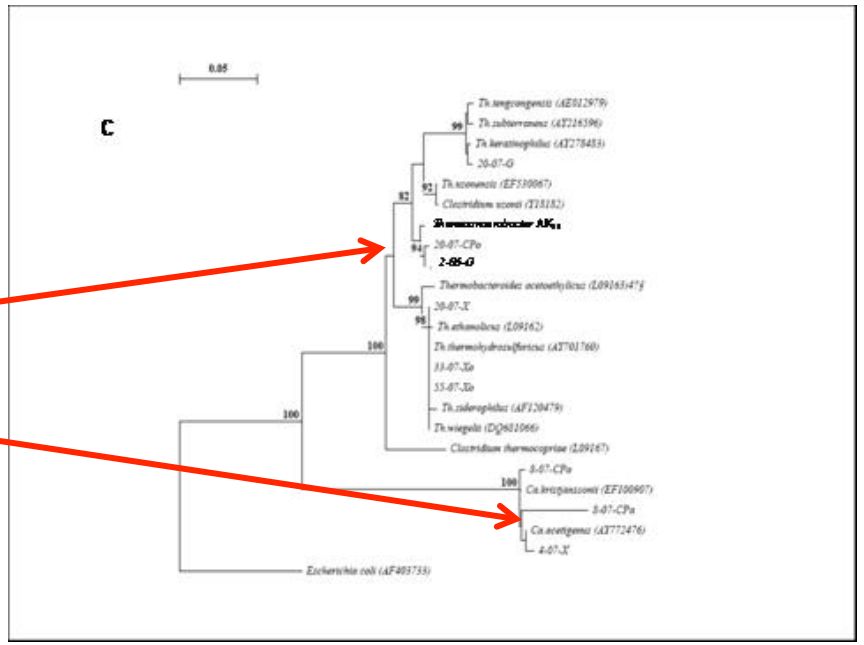
Results

- Low temperature hot springs
  - *Thermoanaerobacterium, Clostridium, Paenibacillus, Caloramator*
  - Sugars → ethanol, butyrate (acetate, H_2)
- High temperature hot springs
  - *Thermoanerobacter , Caldicellulosiruptor,*
  - Sugars → acetate + H_2
- A clear correlation between phylogeny (types of bacteria), temperature and end product formation
- Culture collection obtained



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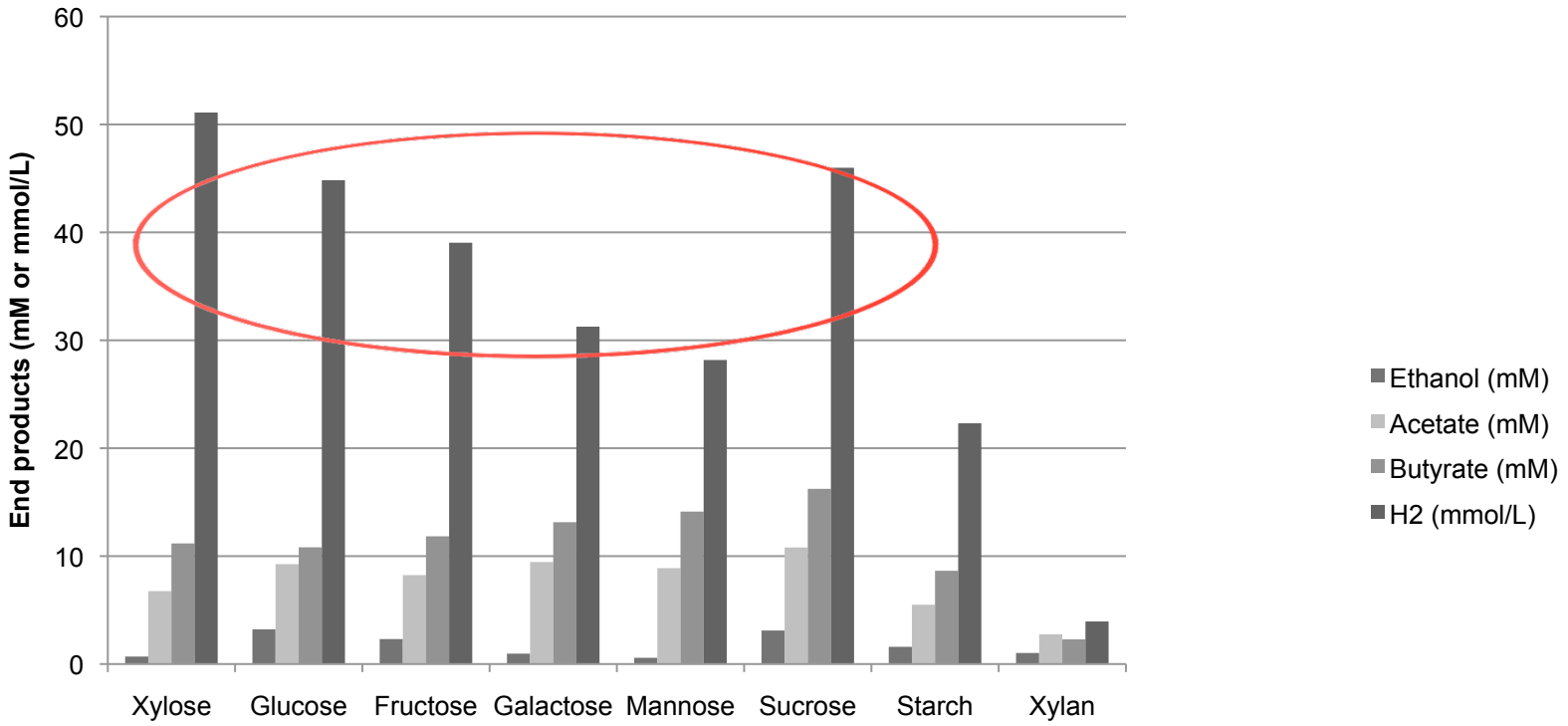
Example of AK14

- A moderate thermophilic bacterium – belongs to *Clostridium*
- Icelandic Agricultural Sciences 2010
- Acetate/butyrate fermentation spectrum
  - End product formation from glucose and xylose (both 20 mM)

20 mM Glucose → 2.8 mM EtOH + 7.5 mM Acetate + 10.7 mM Butyrate + 30.5 mM H_2 + 31.7 mM CO_2

20 mM Xylose → 0.0 mM EtOH + 5.9 mM Acetate + 10.9 mM Butyratre + 35.3 mM H_2 + 27.7 mM CO_2

Substrate spectrum from 20 mM or 3 g/L of various carbohydrates



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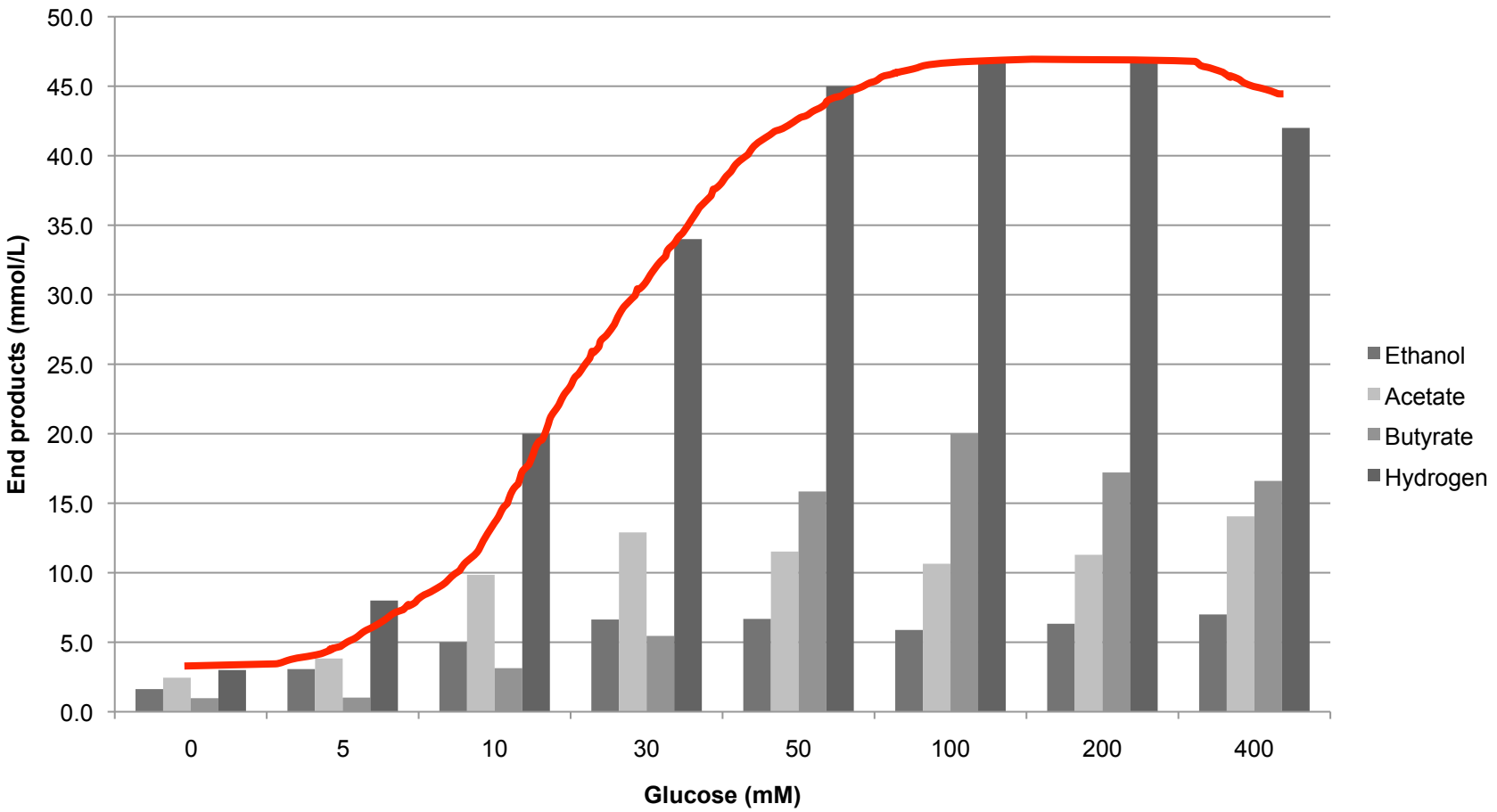
Initial glucose concentrations



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pH_2

- By simply using different gas-to-liquid ratios → get insight into the effects of hydrogen in the gas phase on end product formation and the inhibition of hydrogen production
- Use 20 mM Glucose
- L-G ratios: 3.0 → 0.02
  - Example: 3.0 = 90 mL liquid and 30 mL gas

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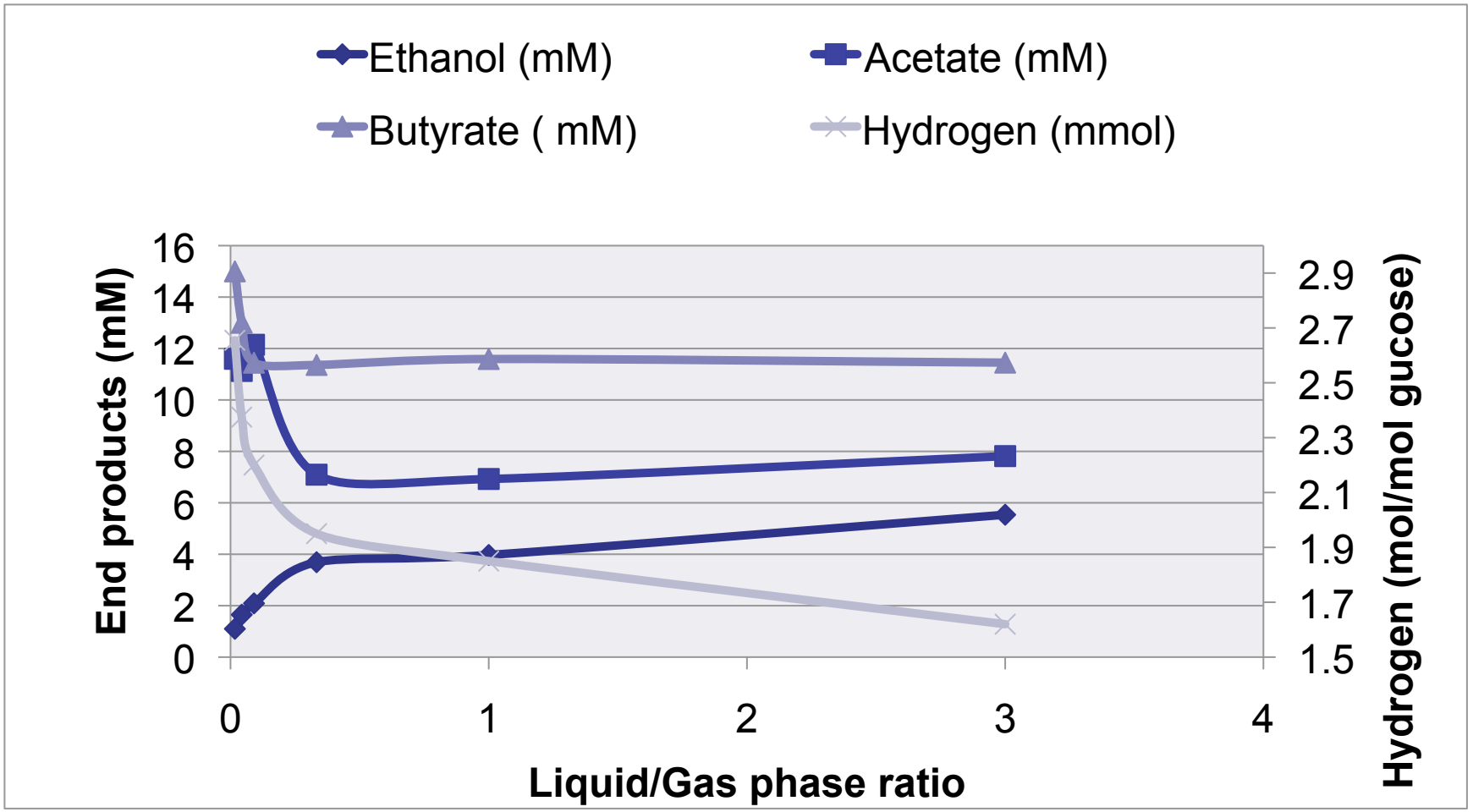
pH_2



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pH_2

- As expected, lower H_2 yields followed the decrease in acetate and butyrate formation as against an increase in ethanol production.
- Using the fermentation data from the lowest and highest L/G ratios the following equations are observed:

1.0 Glucose ◊ 0.06 EtOH + 0.59 Acetate + 0.75 Butyrate + 2.60 $_2$H + 2.15 CO_2 (low L/G (0.05) ; Eq.1)

1.0 Glucose ◊ 0.28 EtOH + 0.39 Acetate + 0.58 Butyrate + 1.60 $_2$H + 1.63 CO_2 (high L/G (3.0) ; Eq.2)

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But....

- .... H_2 production from monosugars is one thing → next step is to go to complex biomass → Production of second generation of hydrogen

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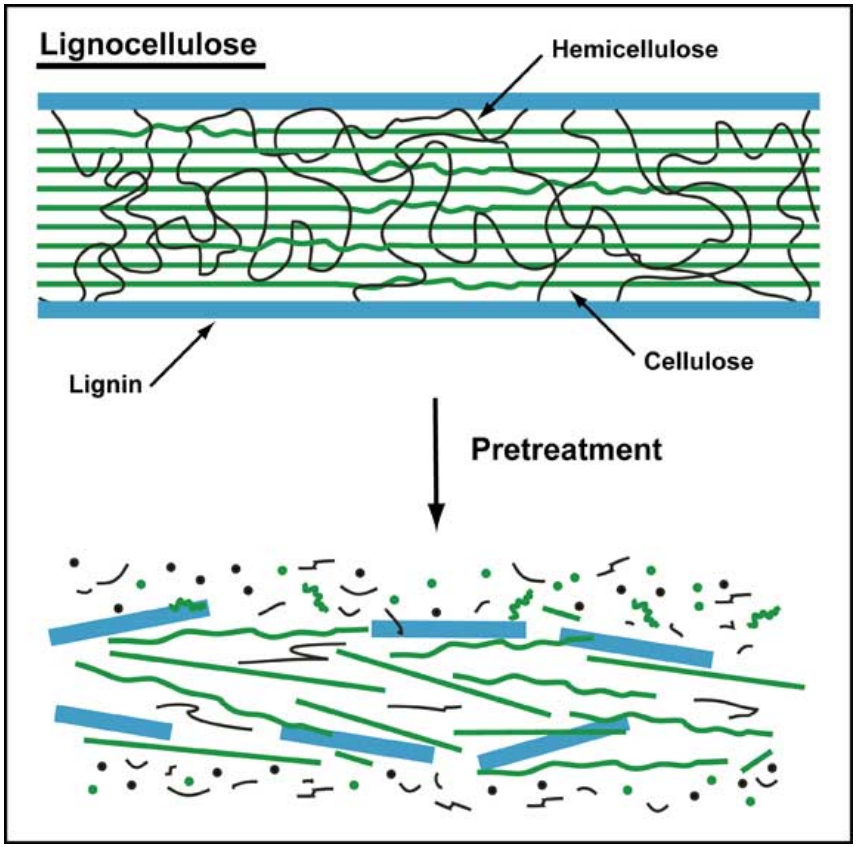
2nd generation – a closer look

- Production of ethanol from waste material
- Lignocellulose
  - Cellulose, hemicellulose and lignin
  - Grass, starw, saw, etc
  - 2° - generation hydrogen production
- Is it possible to produce hydrogen from such biomass?
- The basic structure of lignocellulose is the same, i.e. polymetric sugars that can be converted to monosugars → and ferment to ethanol.
- More expensive, more pretreatment, and enzymes.



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Experimental

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Preparation of HL

- Hydrolysates (HL) were made from different biomasses:
  - Whatman filter paper (cellulose),
  - hemp (*Cannabis Sativa L.*) – leaves and stem fibres
  - newspaper with ink (NPi),
  - barley straw (BS) (*Hordeum vulgare L.*) and
  - grass (*Phleum pratense L.*).
- Chemical pretreatment acid and base
- Heat autoclaving for 30 minutes (121°C).
- Enzymes (Celluclast® and Novozyme 188)
- → Lignocellulosic hydrolysates ready for fermentation

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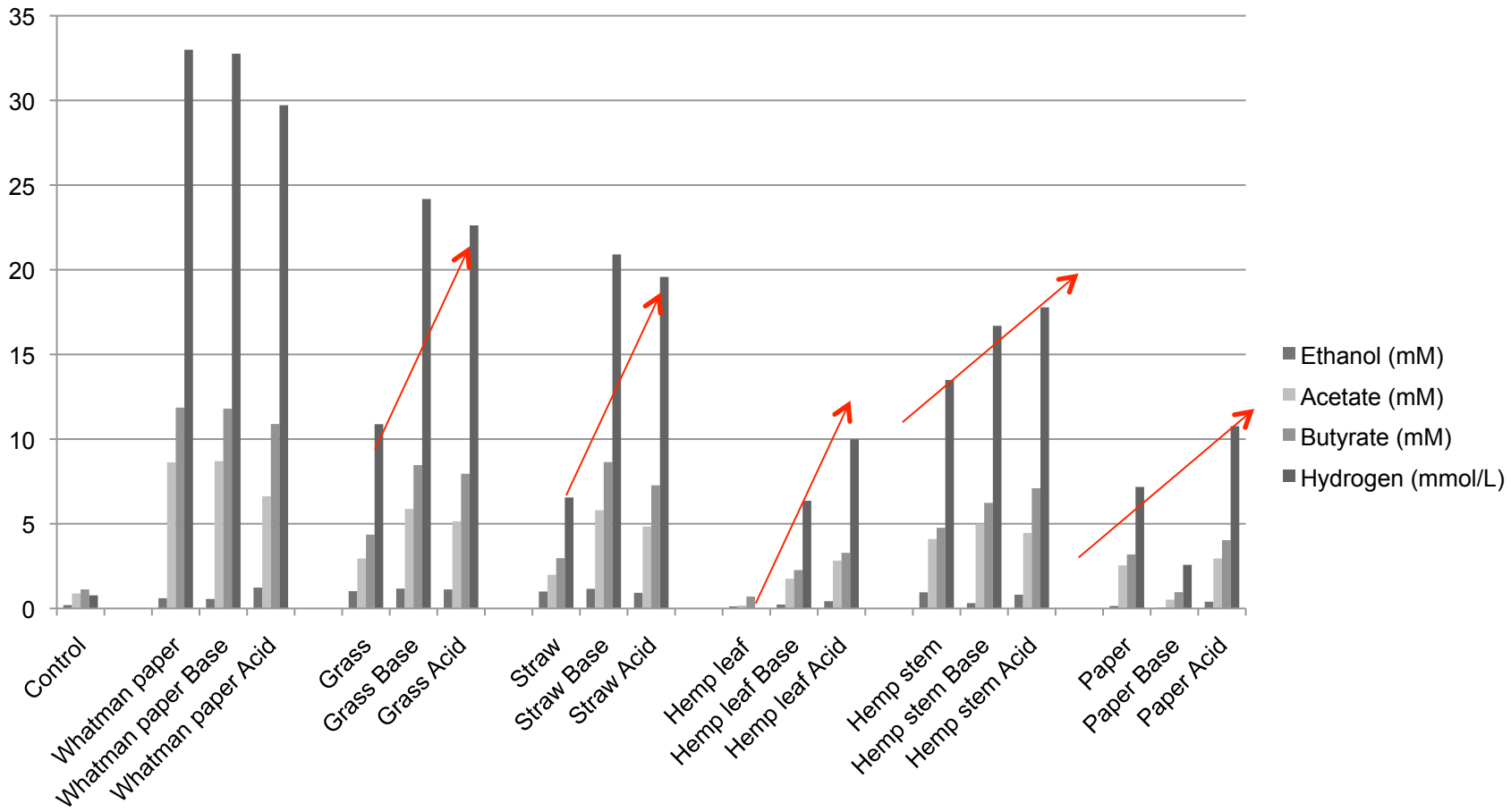
Second generation of H_2



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Second generation of H_2

- The stochiometry for pure glucose and the cellulose hydrolysate (HL) experiments are:

$$1.00\ \text{Glucose} \Diamond\ 0.20\ \text{EtOH} + 0.35\ \text{Acetate} + 0.58\ \text{Butyrate} + 1.60\ H_2 + 1.84\ CO_2\ (\text{glucose})$$

$$1.00\ \text{Glucose} \Diamond\ 0.09\ \text{EtOH} + 0.36\ \text{Acetate} + 0.45\ \text{Butyrate} + 1.78\ H_2 + 1.35\ CO_2\ (\text{HL})$$

- The end product formation in the cellulose hydrolysate experiment was slightly higher except for ethanol and carbon recovery was 80%.
- The hydrogen yield on cellulose hydrolysate was 1.39 mol-H_2/mol-glucose equivalent

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Future aspects - questions

- Initial substrate concentratins for thermophiles seem to be a problem → fed batch or continuous
- Lignocellulose – use waste first !
- Hydrogen removal or use bacteria that tolerate high partial pressures of hydrogen