Bio-hydrogen production

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HYDROGEN AS A CLEAN ENERGY SOURCE

www.energy.gov

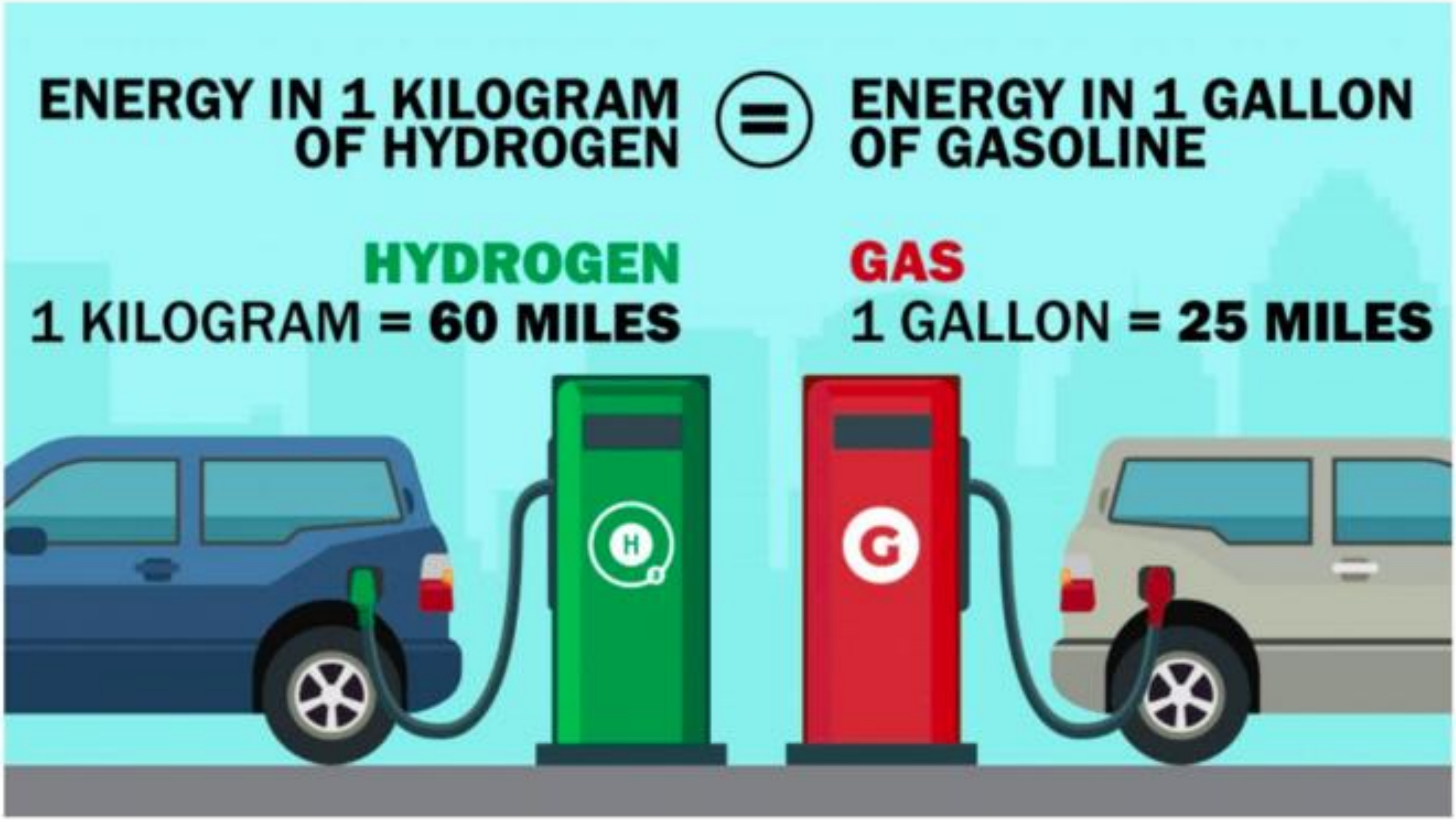
Office of energy efficiency & renewable energy

- Hydrogen can store & deliver clean energy for many uses across economic sectors, including transportation
- It has the potential to reduce air pollution such as greenhouse gases from trucks, buses, planes & ships.
- Greenhouse gases trap heat & contribute to climate change, the transportation sector is responsible for 29% of this emission.

HYDROGEN AS A CLEAN ENERGY SOURCE

- Commercial flights that fly “entirely on hydrogen” planned for 2024 (Anmar Frangoul) CNBC Oct 27 2019.
- For airbuses – hydrogen is promising decarbonisation technologies for aviation.
- Plans to bring a low-carbon commercial aircraft to market by 2035. https://www.airbus.com innovation

Filling Up with Hydrogen



BACTERIAL HYDROGEN PRODUCTION

- Fermentation : a) sakharolytics

  b) proteolytics

- In anaerobic ecosystems following groups of lithotrophic microorganisms are competing for hydrogen:

  1 – SRB

  2 – Acetogenic bacteria

  3 – Methanogenic archaea

- Enzymes of these microorganisms have a very high affinity for hydrogen molecules

BACTERIAL HYDROGEN PRODUCTION

- In nature, H_2 -producers tightly associated with hydrogen-consuming bacteria
- Both, the producers & consumers of H_2, equipped with special enzymes – ***hydrogenases***
  - located in periplasm in Gram- negative bacteria (MBH),
  - Could be soluble (SH)
  - Regulatory hydrogenases (RH)
- For industry, Gram-negative bacteria are preferable since no spore-formation occurs during continuing batch cultivation

BACTERIAL HYDROGEN PRODUCTION

- *Alkalispirochaeta americana* ASpG1^T was isolated from alkaline Mono Lake in California.
- This is a free-living, not pathogenic spirochete requires for growth anaerobic buffer system with pH 9.0-10.0 & 3% NaCl (marine salinity).
- It was isolated as a satellite of the H_2 -consuming SRB *Desulfonatronum thioreducens* MLF1^T
- *In vitro* these bacteria grow better in *binary culture* since SRB removes inhibiting concentrations of H_2 for sugar-lytic spirochete

BACTERIAL HYDROGEN PRODUCTION

- Both these bacteria are gram- negative, not spore-forming with a wide periplasmic space within cell wall
  - Comparison of their's hydrogenase activities may show similarities
  - Also, comparison of genes responsible for these enzymes correspondently may demonstrate the gene transfer (pills were detected on the surface of SRB strain MLF1).
- Some hydrogenases have reversible function of uptake/release hydrogen molecules.
- But others have restricted one direction function.

BACTERIAL HYDROGEN PRODUCTION

- In Mono Lake the source of sugars for ***A. americana*** comes from algae, a photosynthetic alkalophlic cyanobacteria & diatoms – producer of organic matter in the lake ecosystem.

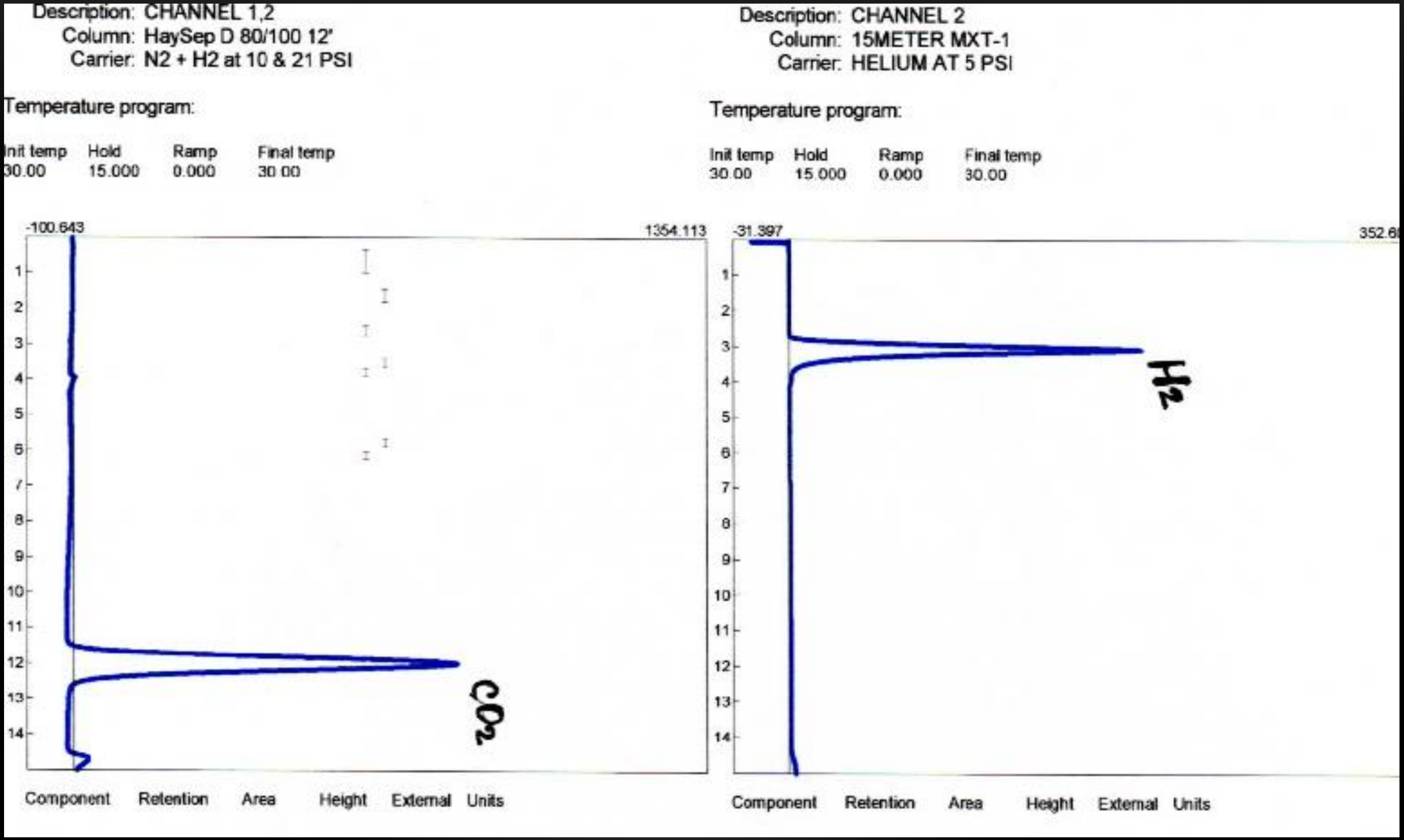
List of substrates supporting *In vitro* growth:

- *D*-glucose D- Ribose
- *D*-fructose D- Arabinose
- Maltose Lactose
- Sucrose Mannose
- *D*- mannitol D- Trehalose
- *Starch*

BACTERIAL HYDROGEN PRODUCTION

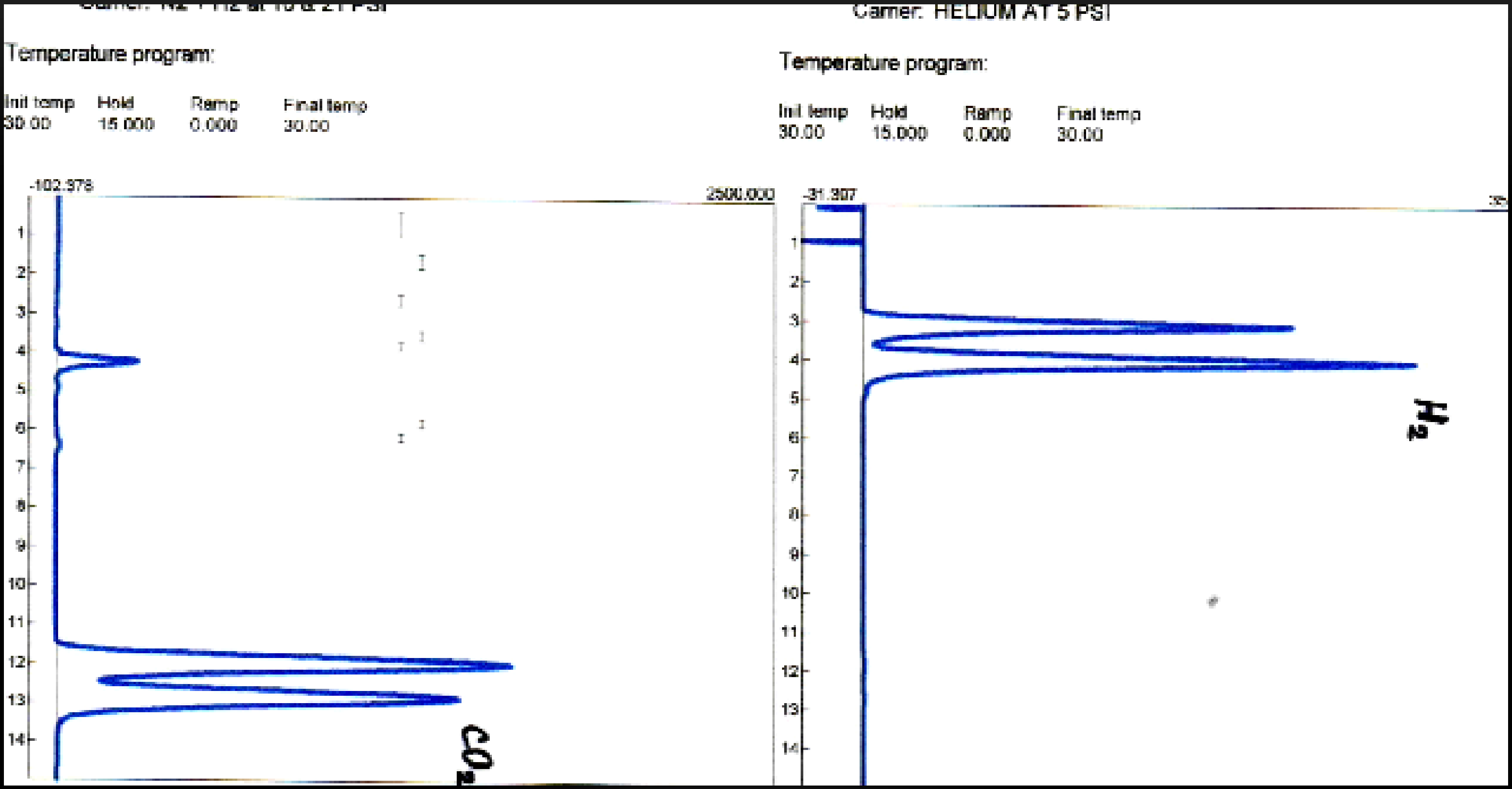
- Photosynthetic algae is a primary producer of organic matter in ecosystem of Mono Lake
- Grown biomass is degraded at a soapy highly mineralized carbonated water where temperature may rich +50-55 ^{o}C in summer
- Products of dissipated algal biomass serve as growth substrates for primary & secondary anaerobes in the trophic chain of the biome
- Enzyme activity of proteo- & sacharo-lytic bacteria accelerates the bioremediation/recycling organic matter
- Final products of fermentation: H_2, CO_2, CO & volatile fatty acids are used as Dē in metabolism of lithotrophic bacteria & archaea

MEASUREMENTS OF HYDROGEN PRODUCTION



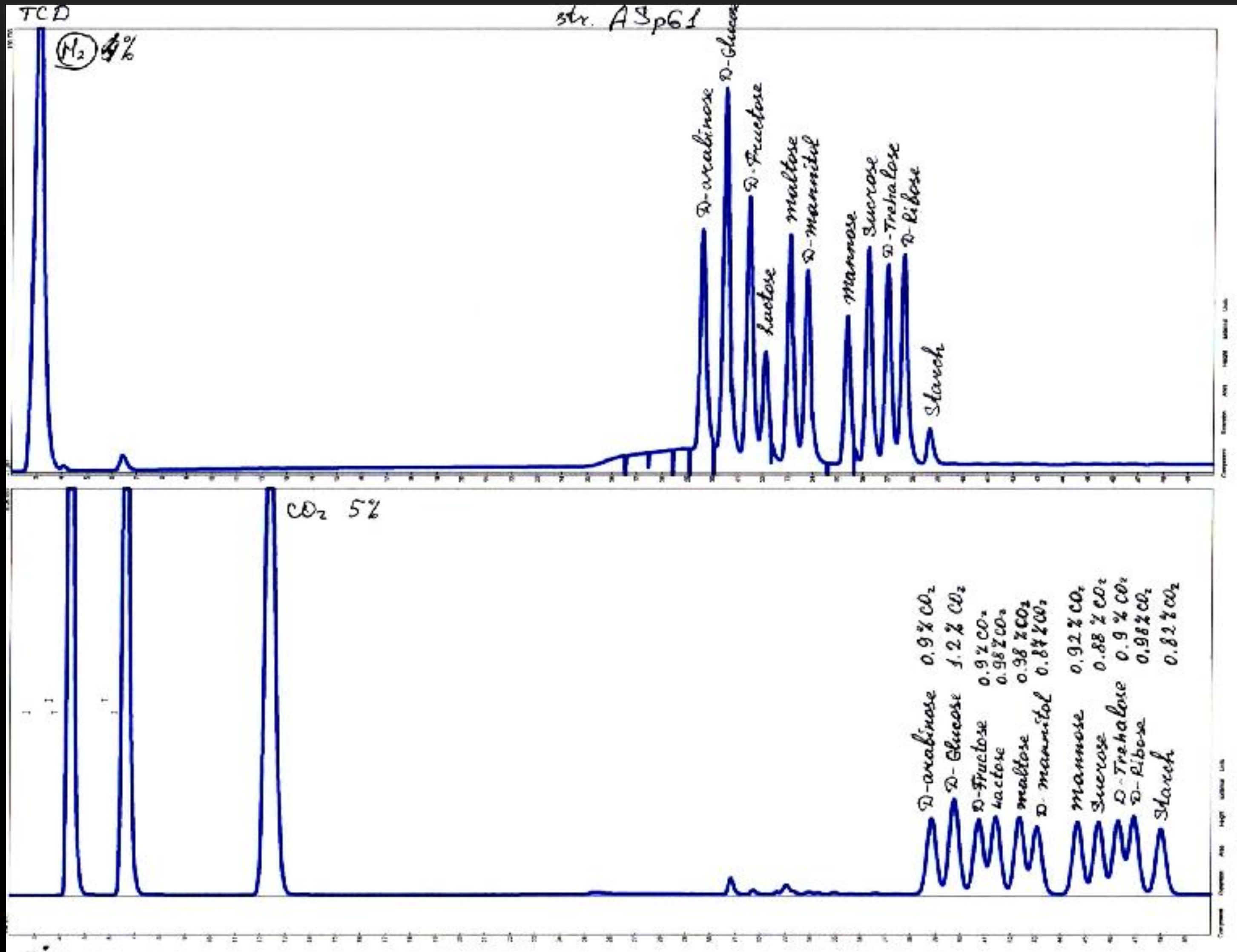
29 hours incubation of strain ASpG1 on *D*-fructose

MEASUREMENTS OF HYDROGEN PRODUCTION



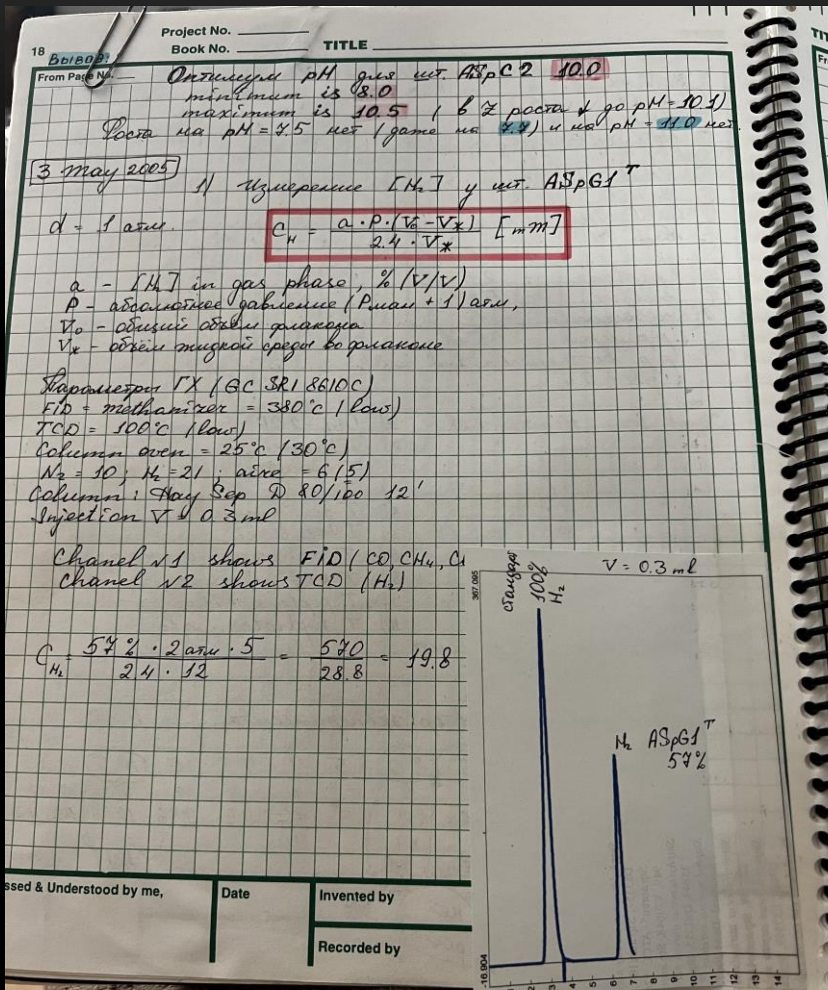
44 hours incubation of strain ASpG1 on *D*-fructose

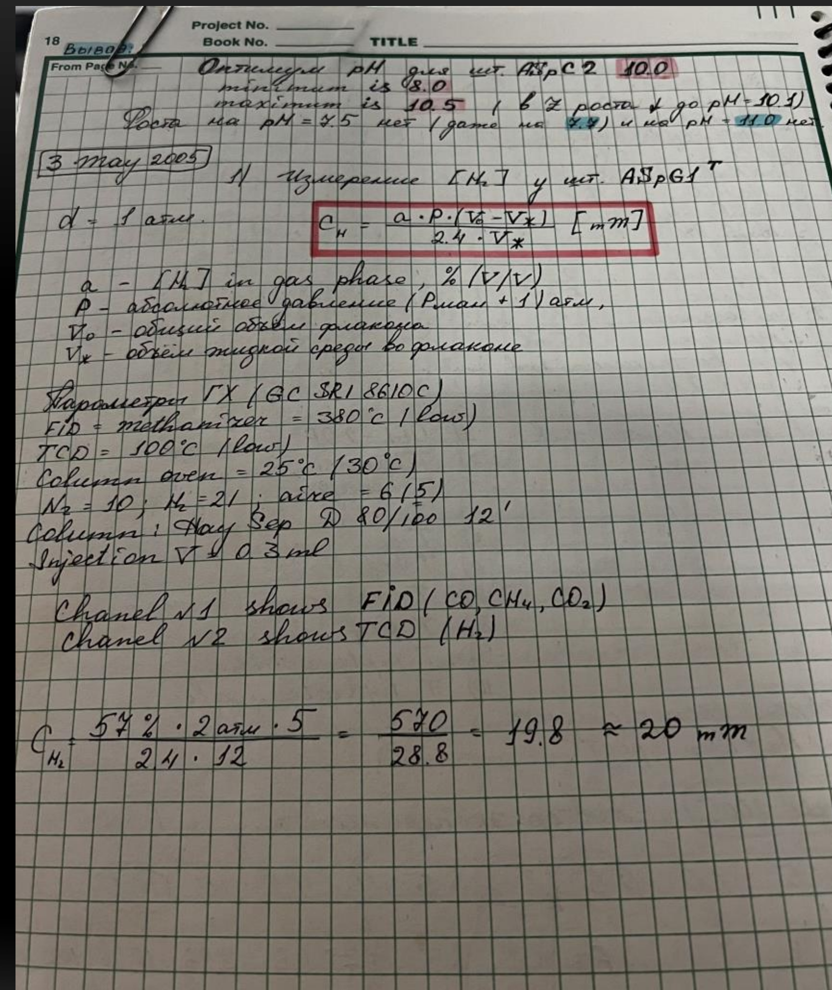
MEASUREMENTS OF HYDROGEN PRODUCTION



The highest H_2- productivity was observed on *D*-glucose

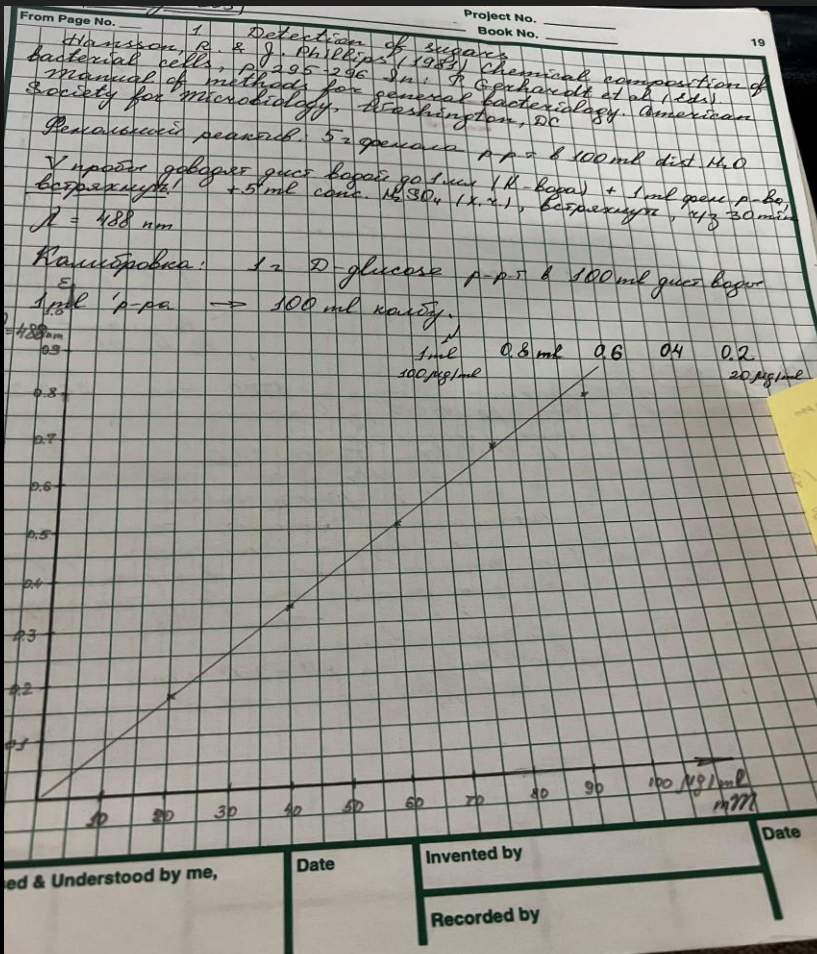
MEASUREMENTS OF HYDROGEN PRODUCTION

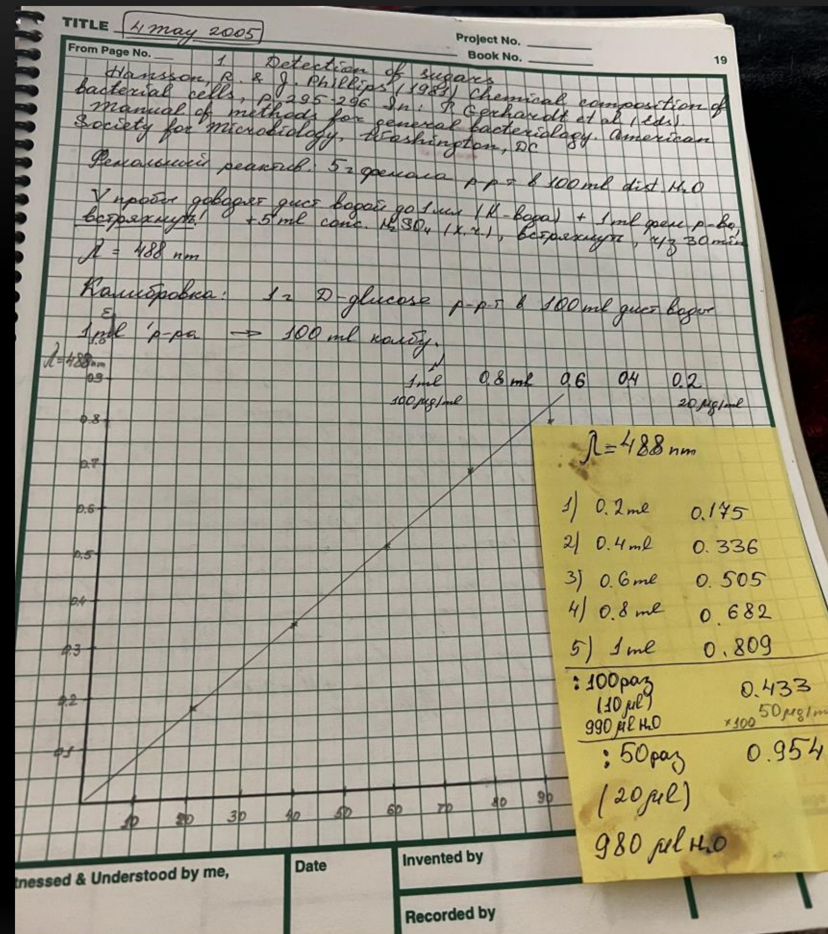




990 g of D-Glucose produced 40 g of H2 in 24-48 hours

MEASUREMENTS OF SUGARS CONSUMPTION





On 1 mol Glucose 4 mol of H_2 are produced (5.5 mM – 20 mM → **1:4**)

BACTERIAL HYDROGEN PRODUCTION

➢ *Conclusions on conducted experiments at Astrobiology Lab:*

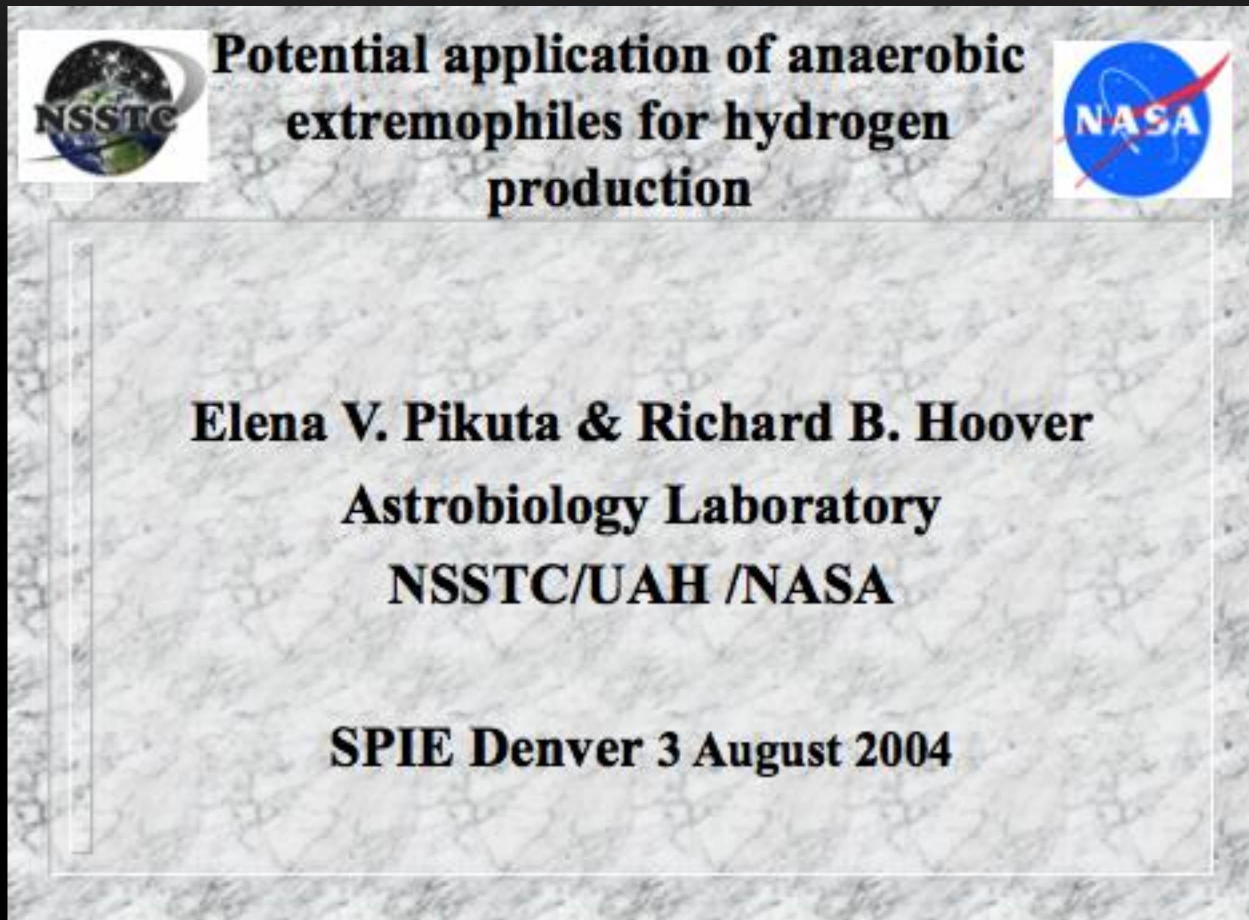
① **the bio-production of H_2 gas could be safely performed at big scale batch cultivation (anaerobic exit of H_2:CO_2 is safe, not explosive gas mixture).**

② **Applied growth medium inhibits development of pathogenic contamination as well as methanogenic archaea.**

③ **The best yield of H_2 was observed on *D-* glucose & *D-* fructose.**

④ **Batch cultivation demonstrated correlation between optic density (along with cell number count) & H_2 gas produced – for estimation.**

BACTERIAL HYDROGEN PRODUCTION



This portion of data was obtained at Astrobiology Lab., NSSTC

Hydrogenases: Classification &physiological functions

Hydrogenase is the key enzyme of catabolism in cell.

- Catalyzes converse reaction of H_2 oxidation: $H_2 = 2H^+ + 2\bar{e}$
- Responsible for consumption & excretion of H_2

- **Were found in *Prokaryotes*** including aerobes, facult. anaerobes, phototrophs & obligate anaerobes (methanogenic, acetogenic, N_2-fixing, SRB & archaea);

- Also **in *Eukaryotes* algae, protozoa & higher plants.**

  - May consume H_2 as energy source, or as ē sink

- In dependence upon Me in active center, they are classified on **FeFe-, NiFe-, NiFeSe- & metal-free hydrogenases.**

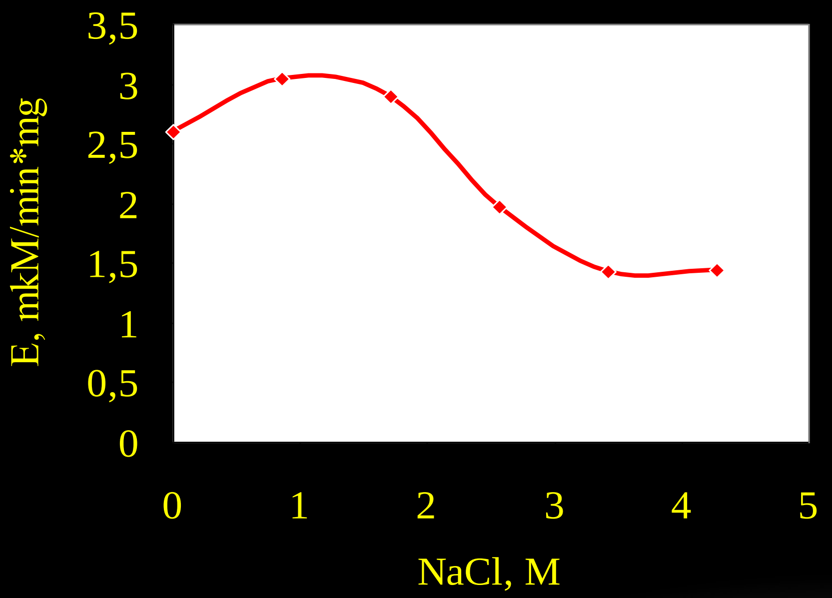
Hydrogenase from haloalkaliphilic, sulfate-reducing bacterium *D. thiodismutans*

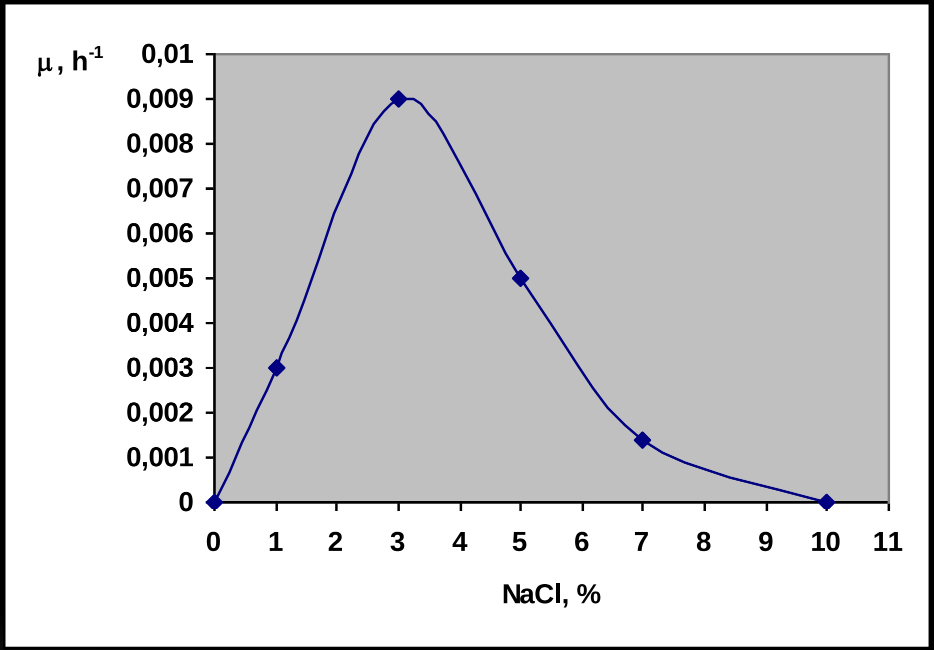
- *D. thiodismutans* is chemolithoautotrophic SRB, capable of growth on H_2 + CO_2 by reduction of SO_4^{2-} to H_2S
- This organism also capable to perform reaction dismutation, or inorganic fermentation.
- The only end catabolic product at growth on formate or H_2 with SO_4-reduction is H_2S
- All these features explain high activity of hydrogenase & it's resistance to high pH & salinity.

Gram-negative stained cells of *D. thiodismutans* MLF1 & transmission electron microscope image of vibrion-shaped, cell with polar flagella

Hydrogenase of *Desulfonatronum thiodismutans*

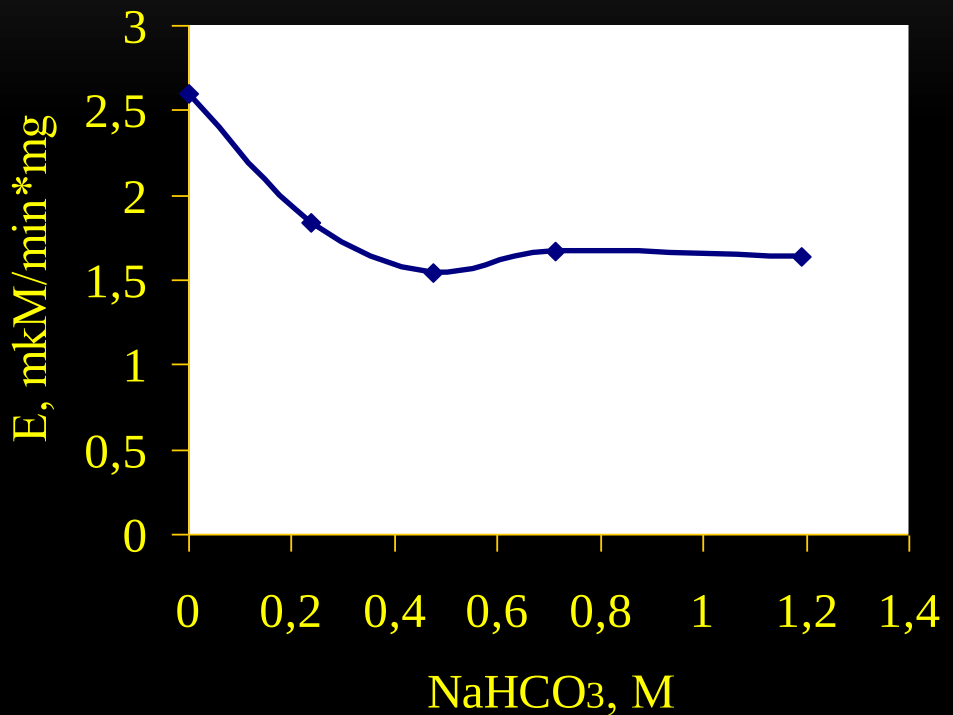
- Physiological optima & ranges of this organism (right) were significantly smaller then functional enzyme (left):





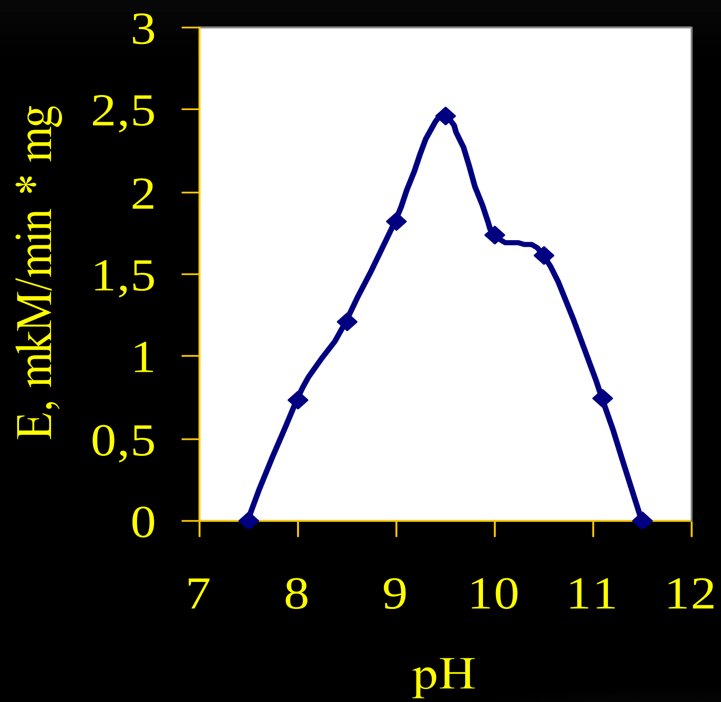
Dependence of hydrogenase activity of *D. thiodismutans* upon NaCl

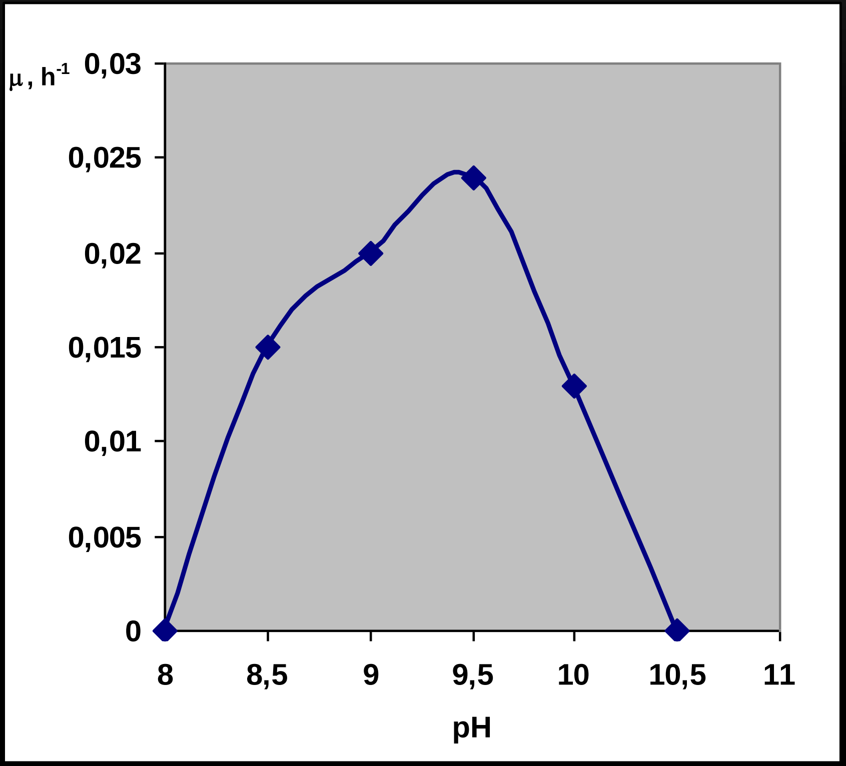
Hydrogenase of *Desulfonatronum thiodismutans*



Dependence of hydrogenase activity of *D. thiodismutans* upon $NaHCO_3$

HYDROGENASE of *D. thiodismutans* MLF1[T]





Dependence of hydrogenase activity of D. thiodismutans upon pH

CONCLUSIONS

- High activity of H_2-oxidizing hydrogenase of *D. thiodismutans* indicates the enzyme performs catabolic function by participation in dissimilatory SO_4-reduction.
- The hydrogenase of *D. thiodismutans* is tolerant to high concentrations of sodium salts. It suggests that this bacterium is physiologically adapted to osmotic stress by high salt concentrations at the expense of enzymes.
- The hydrogenase able to function at high pH that cause adaptation of the bacterium to highly alkaline media.
- All these features suggest this enzyme is a unique subject for diverse biochemical research, and define the potential for biotechnological application.

Salt tolerant & high pH resistant hydrogenase from haloalkaliphilic, sulfate-reducing bacterium *Desulfonatronum thiodismutans*

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BACTERIAL HYDROGEN PRODUCTION

- Future development:

1) Genome study genes associated with hydrogenases to determine if hydrogen production can be improved
2) Measurements of enzyme activity of hydrogenases for increasing yield of $H_{2.}$
3) Work with engineering and gas equipment development for continuing and batch cultivations on big scale.
4) Material science may develop a novel sponge-like materials for absorption and storage of H_2 in small portions with consequent slow release for engine (safe not explosive technology).

BACTERIAL HYDROGEN PRODUCTION

- Department of Energy Joint Genome Institute (JGI) performed draft sequencing under number:

IMG ID 2706795025 – *Alkalispirochaeta americana ASpG1*
GOLD ID Go0097259 *Desulfonatronum thiodissmutans* MLF1

In annotations of the ASpG1 sequence:

- EC:1.12.1.3 Hydrogen dehydrogenase NADP(+)
- Gene 2708603103
- Gene 2708603104

$$H_2 + NADP \longleftrightarrow H^+ + NADP(H^+)$$

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