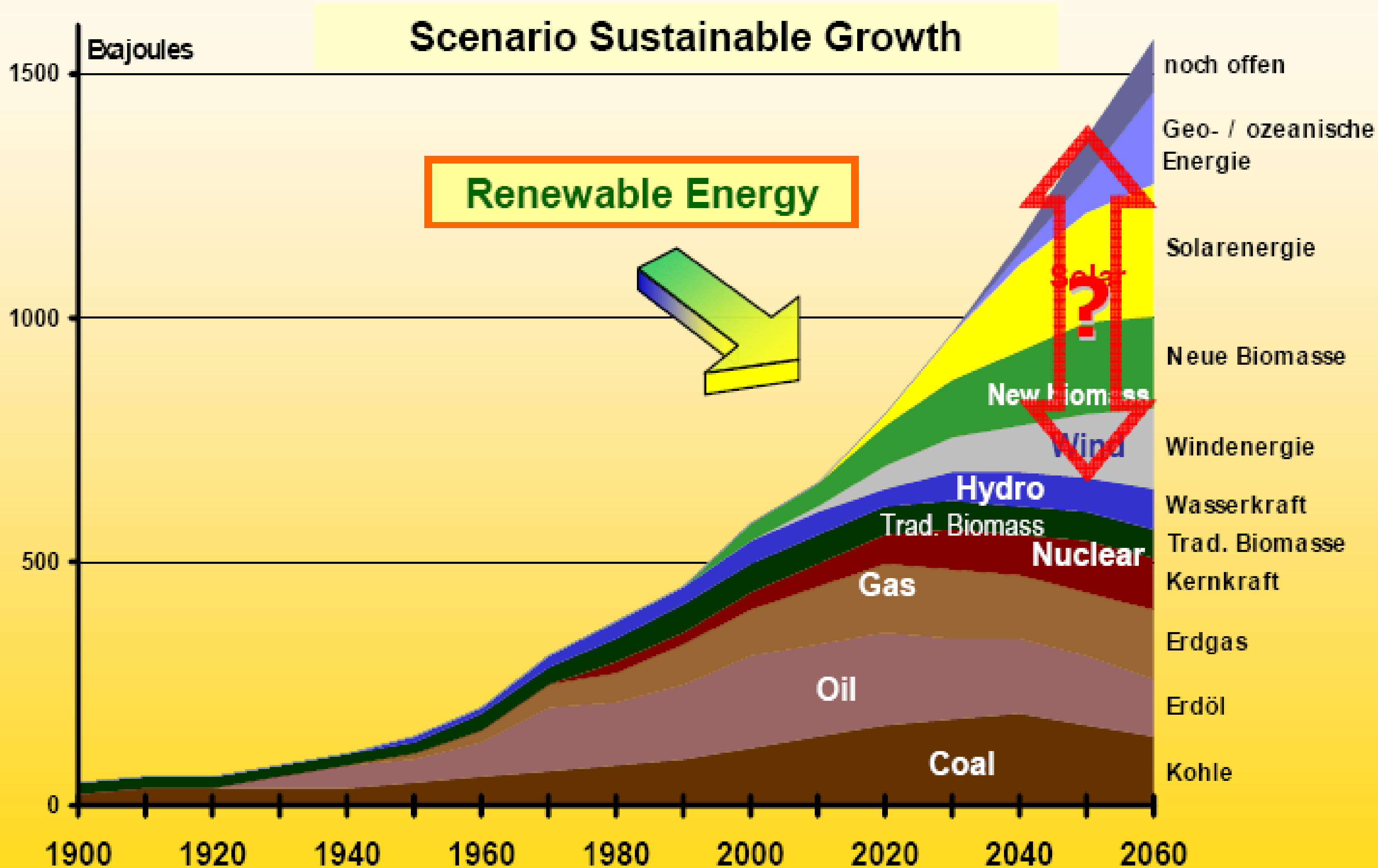
Production of Biofuels

AO Olaniran

Department Microbiology
University of KwaZulu-Natal (Westville Campus)

Deutsche Shell Aktiengesellschaft

World Energy Consumption until 2060



exa = 10^{18} 1 Exajoule = 34,12 Mio t SKE

Quelle: Deutsche Shell AG

- Microorganisms may be used to convert waste products, plants, or microbial biomass into liquid or gaseous fuels
- They can also be used to convert solar energy into biomass that can be fermented to yield fuels

Biofuels crops & products

Oil crops → oils → biodiesal

Grain, Sugar crops → starches sugars → bioethanol

Transport **First generation biofuels**

Transport **Second generation biofuels**

Perennial biomass crops → lignocellulose → liquid fuels

Perennial biomass crops → biomass → heat, Electricity, CHP

Heat and power

Potential molecules for liquid biofuels to replace petrol

- Methanol
- Ethanol
- Butanol
- Hydrogen
- Biodiesel

Ethanol

- Ethanol or ethyl alcohol (C_2H_5OH) is a clear colourless liquid
- It is biodegradable
- low in toxicity and causes little environmental pollution if spilt
- Ethanol burns to produce carbon dioxide and water and is a high octane fuel and can replace lead as an octane enhancer in petrol

- The ability of microorganisms, especially yeasts, to produce ethanol by the fermentation of carbohydrate-containing materials is well known
- The microbial formation of ethanol is used in the production of many beverages:
- ethanol can also serve as a valuable fuel resource
- The addition of ethanol to gasoline can greatly extend this petroleum-based fuel

- Gasoline-ethanol mixtures are commonly known as gasohol
- The normal ratio of gasoline to ethanol is 9:1
- Gasohol can be used directly in present internal combustion automobile engines without any engineering modifications
- It is an efficient fuel, lowering the release of atmospheric hydrocarbon pollutants compared to petroleum

Ethanol use as motor fuel is as old as the automotive industry is

Henry Ford, pure ethanol car (1896)

Ethanol Crops

- The main sources of sugar required to produce ethanol come from **fuel or energy crops**.
- These crops include corn, maize and wheat crops, waste straw, willow and popular trees, sawdust, reed canary grass, cord grasses, jerusalem artichoke, myscanthus and sorghum plants.
- Use of municipal solid wastes to produce ethanol fuel ???

- The extensive production of ethanol requires a large supply of carbohydrate substrates.
- The production of substrates suitable for conversion to fuels such as ethanol and methane involves the conversion of solar energy to plant or microbial biomass and/or the utilization of organic wastes.

- The chemical composition of the substrate is critical in determining whether it can be converted to ethanol efficiently
- Ethanol is normally produced from **simple carbohydrates** by yeasts
- The yeasts used in commercial production are unable to attack complex polymers
- It is usually necessary initially to degrade plant polymers with enzymes produced by the plant itself or by microorganisms other than yeasts

Bioethanol Production

- Ethanol can be produced from biomass by the hydrolysis and sugar fermentation processes.
- Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi cellulose and lignin.
- In order to produce sugars from the biomass, the biomass is pre-treated with acids or enzymes in order to reduce the size of the feedstock and to open up the plant structure.

- The cellulose and the hemi cellulose portions are broken down (hydrolysed) by enzymes or dilute acids into sucrose sugar that is then fermented into ethanol.

- The lignin which is also present in the biomass is normally used as a fuel for the ethanol production.

- Three principal methods of extracting sugars from biomass:
  - concentrated acid hydrolysis
  - dilute acid hydrolysis
  - enzymatic hydrolysis

- The chemical reaction is shown below:

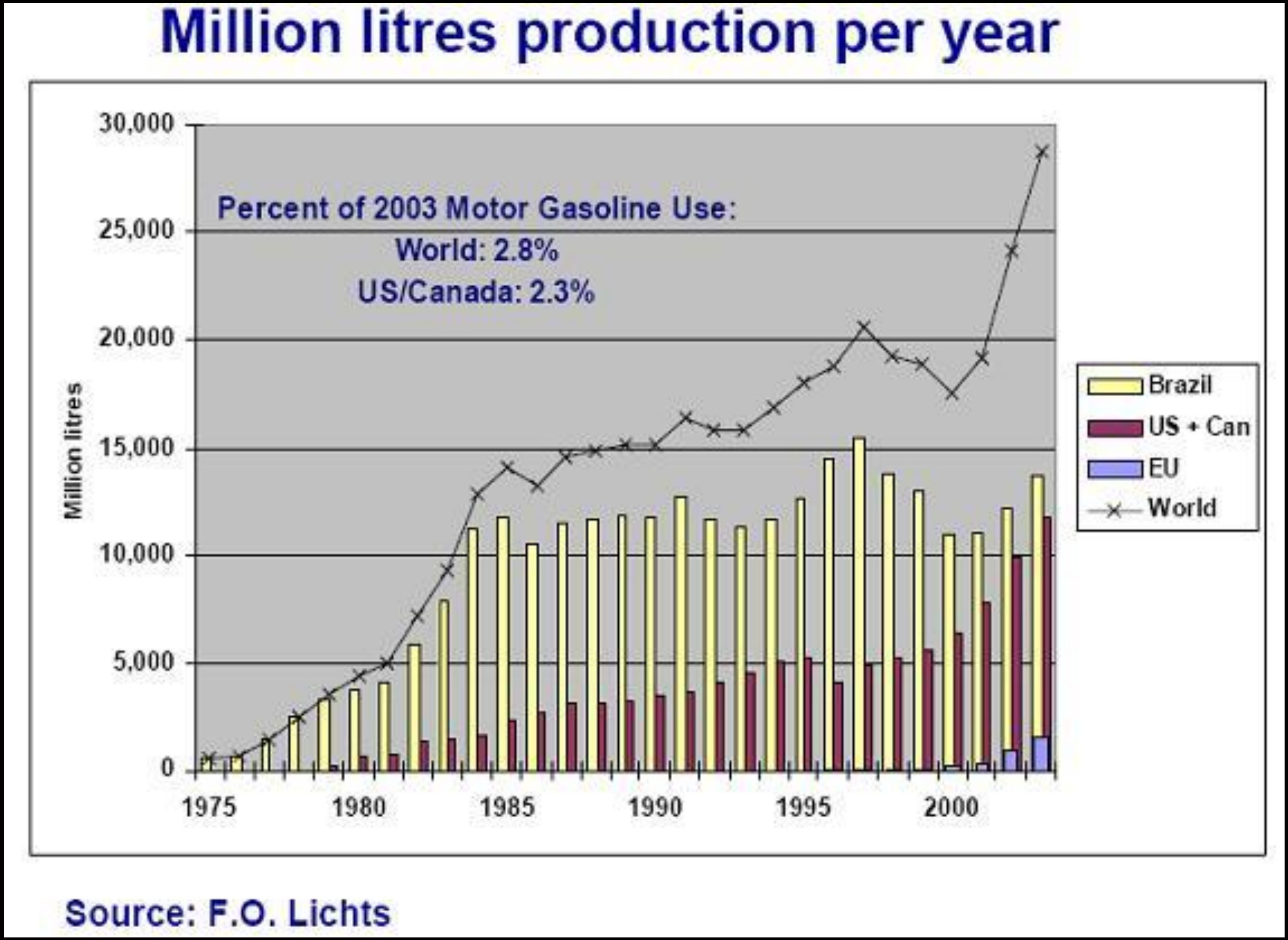
			Invertase			
$C_{12}H_{22}O_{11}$	+	H_2O	→	$C_6H_{12}O_6$	+	$C_6H_{12}O_6$
Sucrose		**Water**	**Catalyst**	**Fructose**		**Glucose**

- The fructose and glucose sugars then react with another enzyme called zymase, which is also contained in the yeast to produce ethanol and carbon dioxide.

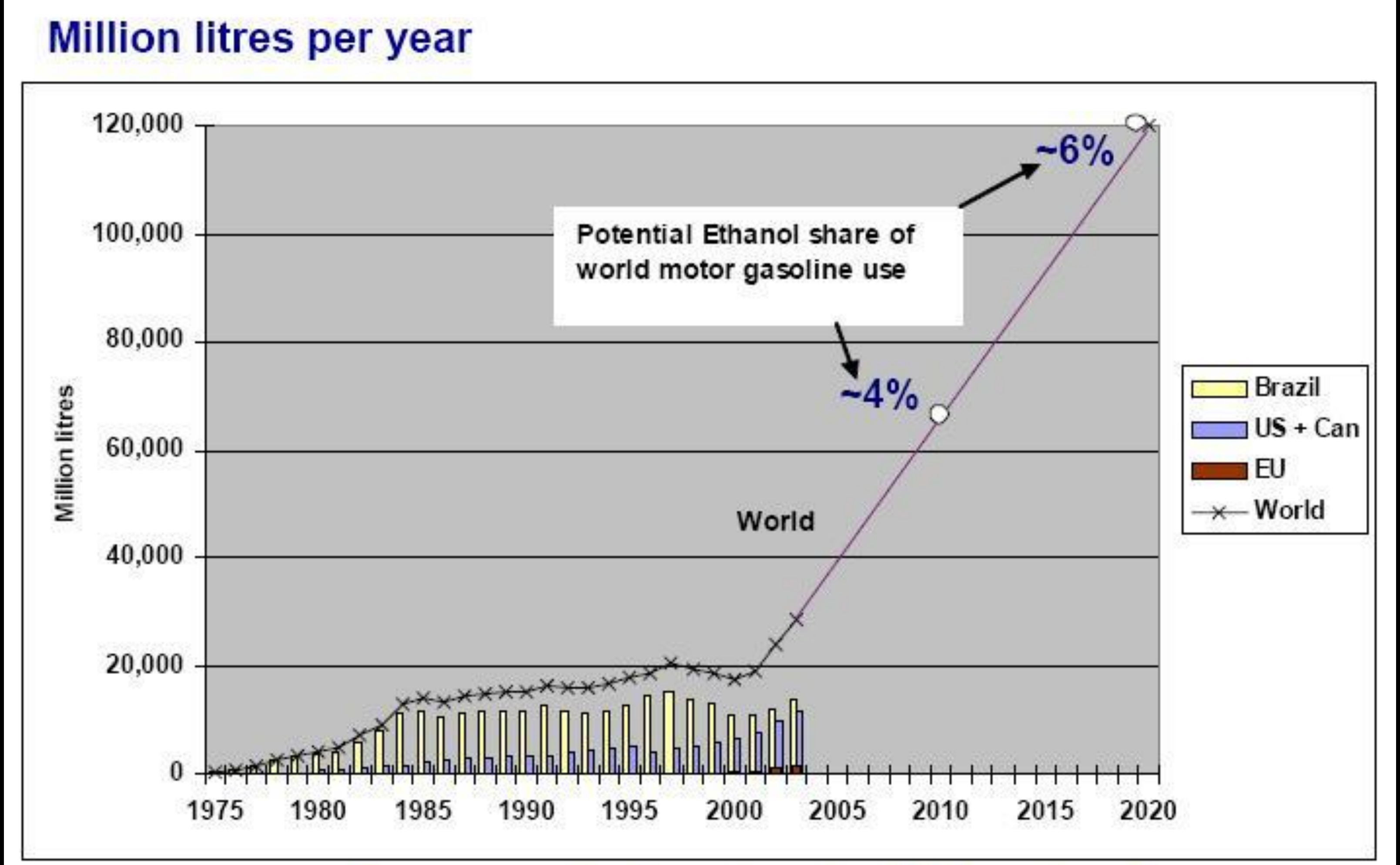
	Zymase			
$C_6H_{12}O_6$	→	$2C_2H_5OH$	+	$2CO_2$
Fructose / Glucose	**Catalyst**	**Ethanol**		

- The fermentation process takes around three days to complete and is carried out at a temperature of between 25°C and 30°C.
- **Fractional distillation** process is used to remove water from the ethanol which is produced
- The distillation process works by boiling the water and ethanol mixture.
- Ethanol has a lower boiling point (78.3°C) compared to that of water (100°C) & turns into the vapour state before the water
- It can be condensed and separated

WORLD ETHANOL PRODUCTION



WORLD ETHANOL PRODUCTION



Source: projections based on IEA review of recent policy initiatives around the world

Advantages of ethanol

- It comes from a renewable resource (i.e. crops like cereals, sugar beet and maize).
- Reduction of greenhouse gas emissions: The road transport network accounts for 22% of all greenhouse gas emissions. The fuel crops absorb the CO_2 they emit through growing.
- Ensures greater fuel security, avoiding heavy reliance on oil producing nations.

- Boost to the rural economy from growing the necessary crops
- Bioethanol is also biodegradable and far less toxic than fossil fuels
- Use of bioethanol in older engines can help reduce the amount of carbon monoxide produced by the vehicle
- Ease of integration into the existing road transport fuel system: In quantities up to 5%, bioethanol can be blended with conventional fuel without the need of engine modifications
- Can replace methyl tertiary butyl ether (MBTE) as an octane booster

Ethanol is not a prefect replacement

- Ethanol has 30% less energy per litre than petrol
- Bioethanol currently is produced from food crops leading to price increases
- Government support is needed to initiate or sustain production
- More than 10% in petrol requires modification to the car fuel system
- Unlikely ever to replace petrol completely

Methane

- Methane fermentation is a versatile biotechnology capable of converting almost all types of polymeric materials to methane and carbon dioxide under anaerobic conditions

- This is achieved as a result of the consecutive biochemical breakdown of polymers to methane and carbon dioxide

- It involves a variety of microorganisms including;

- fermentative microbes (**acidogens**);
- hydrogen-producing, acetate-forming microbes (**acetogens**); and
- methane-producing microbes (**methanogens**)

- The MOS harmoniously grow and produce reduced end-products.

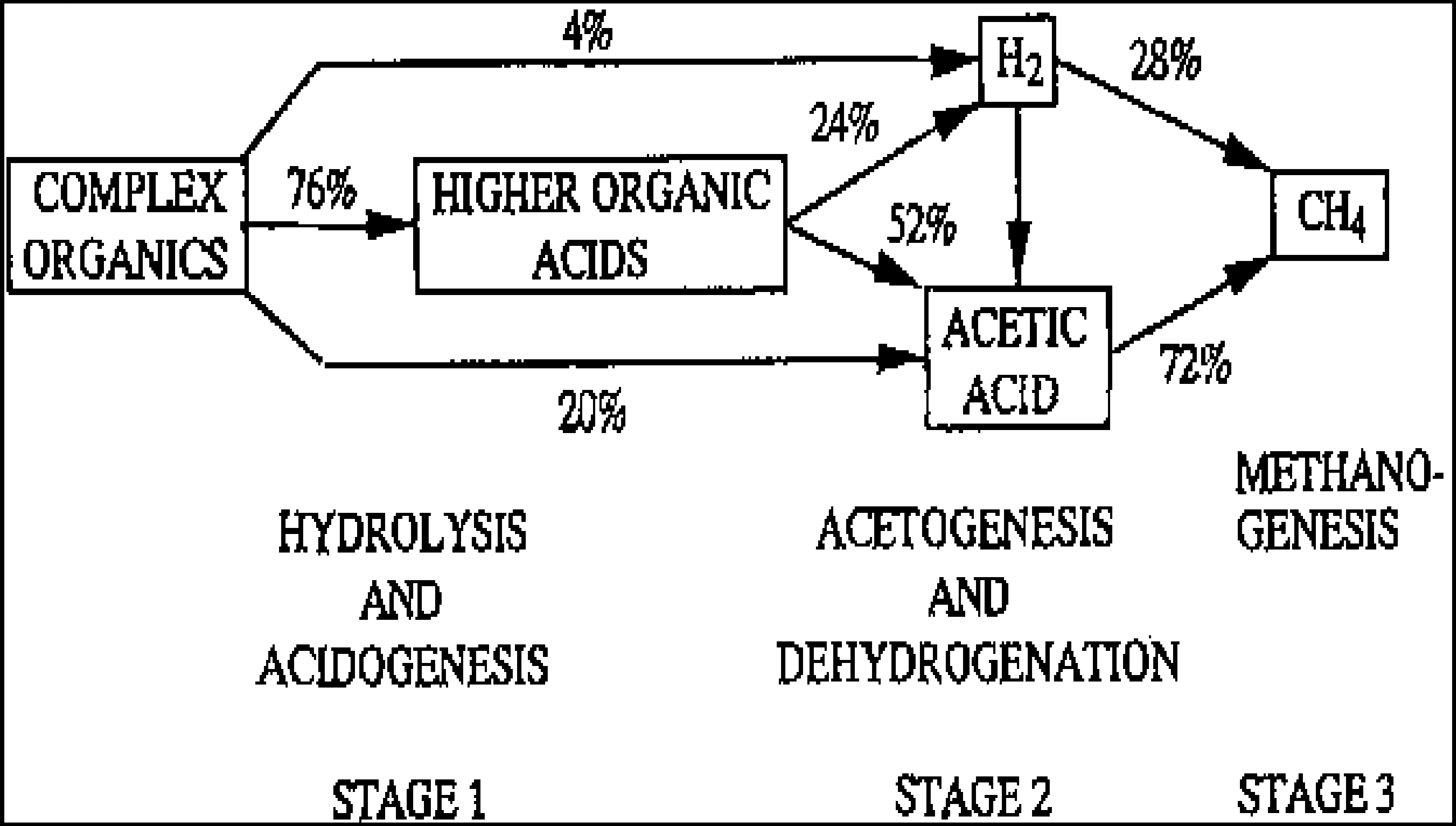
- Anaerobes play important roles in establishing a stable environment at various stages of methane fermentation.

- A description of microorganisms involved in methane fermentation, based on an analysis of bacteria isolated from sewage sludge digesters and from the rumen of some animals, is summarized below:

- The first group of MOS secrete enzymes which **hydrolyze polymeric materials to monomers** such as glucose and amino acids, which are **subsequently converted to higher volatile fatty acids, H_2 and acetic acid** (stage 1).

- In the second stage, hydrogen-producing acetogenic bacteria **convert the higher volatile fatty acids *e.g.,* propionic and butyric acids, produced, to H_2, CO_2, and acetic acid**.

- Finally, the third group, methanogenic bacteria **convert H_2, CO_2, and acetate, to CH_4 and CO_2**.



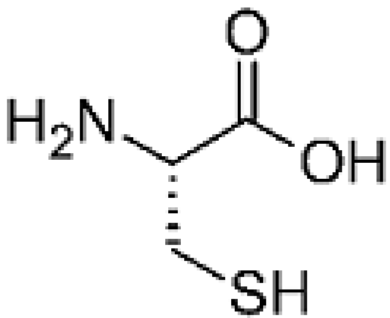
Stages of methane fermentation *Source: McCarty, P.L., (1982)*

Hydrolysis and acidogenesis

- Polymeric materials such as lipids, proteins, and carbohydrates are primarily hydrolyzed by extracellular, hydrolases, excreted by microbes
- Lipases convert lipids to long-chain fatty acids.
- A population density of 10^4 - 10^5 lipolytic bacteria per ml of digester fluid has been reported to be involved.
- **Clostridia** and the **Micrococci** appear to be responsible for most of the extracellular lipase production.

- Proteins are generally hydrolyzed to amino acids by proteases, secreted by *Bacteroides, Butyrivibrio, Clostridium, Fusobacterium, Selenomonas,* and *Streptococcus*.

- The amino acids produced are then degraded to fatty acids such as acetate, propionate, and butyrate, and to ammonia as found in *Clostridium, Peptococcus, Selenomonas, Campylobacter,* and *Bacteroides*.



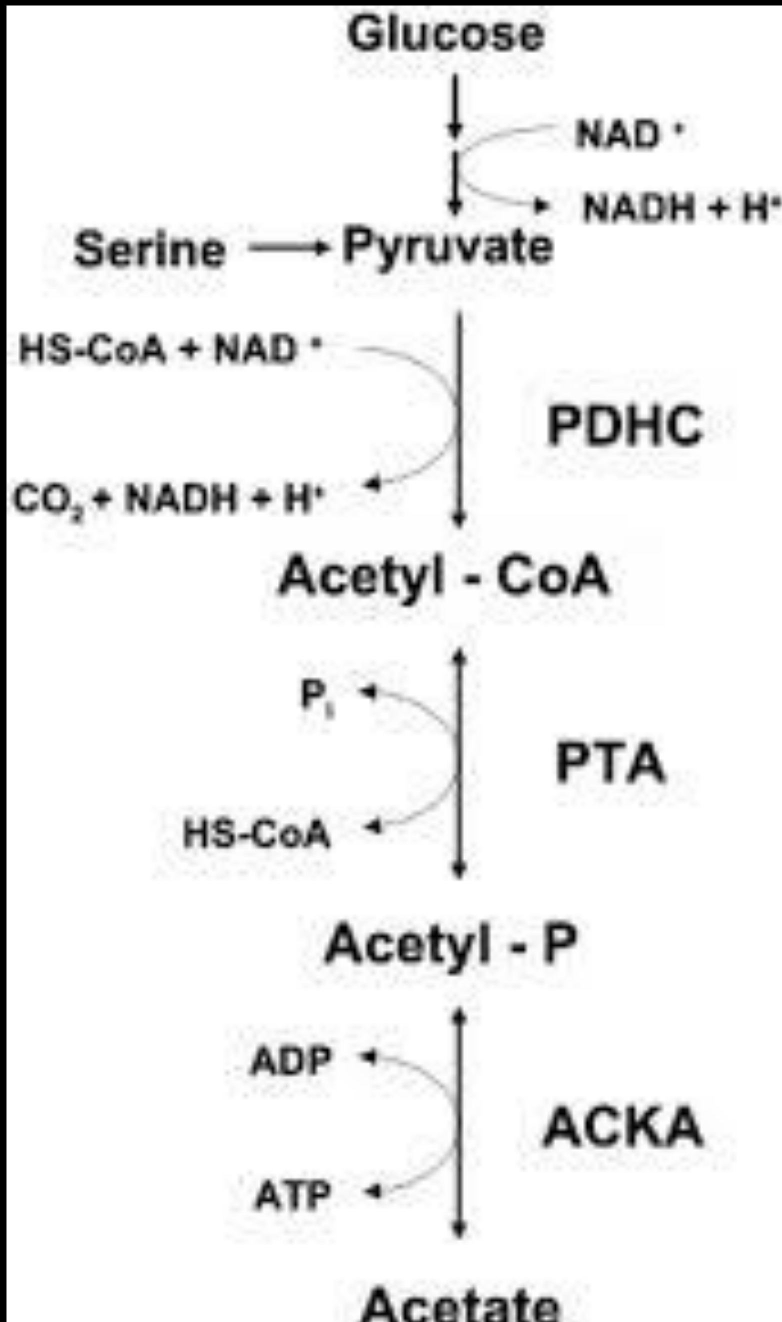
Cysteine

$NaS \rightarrow Na^{+} + S^{2-}$

$Pb(CH_3COO^{-})_2 + S^{2-} \rightarrow PbS\downarrow + 2CH_3COO^{-}$

- Polysaccharides such as cellulose, starch, and pectin are hydrolyzed by cellulases, amylases, and pectinases.
- The majority of microbial cellulases are composed of three species: (a) endo-(β-l,4-glucanases; (b) exo- β-l,4-glucanases; (c) cellobiase or β -glucosidase
- These three enzymes act synergistically on cellulose effectively hydrolyzing its crystal structure, to produce glucose
- Pectins are degraded by pectinases, including pectin esterases and depolymerases.
- Xylans are degraded with α-endo-xylanase and α-xylosidase to produce xylose.

- Sugars, amino acids, and fatty acids produced by microbial degradation of biopolymers are successively metabolised by groups of bacteria
- They are primarily fermented to acetate, propionate, butyrate, lactate, ethanol, carbon dioxide, and hydrogen



Glucose
NAD⁺
NADH + H⁺
Serine
Pyruvate
HS-CoA + NAD⁺
PDHC
CO_2 + NADH + H⁺
Acetyl - CoA
P_i
PTA
HS-CoA
Acetyl - P
ADP
ACKA
ATP
Acetate

Acetogenesis and dehydrogenation

- Some acetate (20%) and H_2 (4%) are directly produced by acidogenic fermentation of sugars, and amino acids
- However, both products are primarily derived from the acetogenesis and dehydrogenation of higher volatile fatty acids (Stage 2)
- **Obligate H_2-producing acetogenic bacteria are capable of producing acetate and H_2 from higher fatty acids**

- Only *Syntrophobacter wolinii,* a propionate decomposer and *Sytrophomonos wolfei,* a butyrate decomposer have thus far been isolated.

- This is due to technical difficulties involved in the isolation of pure strains

- H_2 produced severely inhibits the growth of these strains.

Overall breakdown reactions for long-chain fatty acids

- **Even-numbered**

$$CH_3CH_2CH_2COO^- + 2\ H_2O \leftrightarrow 2\ CH_3COO\text{-} + 2H_2 + H^+$$

- **Odd-numbered**

$$CH_3CH_2CH_2CH_2COO^- + 1\ H_2O \leftrightarrow CH_3CH_2COO^- + CH_3COO^- + 2\ H_2 + H^+$$

- **Branched-chained**

$CH_3CHCH_2CH_2CH_2COO^-$
|
CH_3 $\quad + 2\ H_2O \leftrightarrow CH_3CHCH_2COO^- + CH_3COO^- + 2H_2 + H^+$
|
CH_3

Methanogenesis

- Methanogens are physiologically united as methane producers in anaerobic digestion (Stage 3).
- Although acetate and H_2/CO_2 are the main substrates available in the natural environment, formate, methanol, methylamines, and CO are also converted to CH_4.
- Methanogens can be divided into two groups: **H_2/CO_2**- and **acetate-consumers**.
- Examples are *Methanosarcina* spp. and *Methanothrix* spp.

- H_2/CO_2-consuming methanogens reduce CO_2 as an electron acceptor via the formyl, methenyl, and methyl levels through association with unusual coenzymes, to finally produce CH_4
- The overall acetoclastic reaction can be expressed as:
- $CH_3COOH \longrightarrow CH_4 + CO_2$
- Since a small part of the CO_2 is also formed from carbon derived from the methyl group, it is suspected that the reduced potential produced from the methyl group may reduce CO_2 to CH_4 (Thauer *et al.*, 1989).

Hydrocarbons Other Than Methane

- Hydrocarbon biosynthesis occurs in various algae, fungi, and bacteria, such as the bacterium *Micrococcus luteus*
- Some bacteria, Fungi, and algae synthesize significant levels of hydrocarbons
- This suggests the possibility that microorganisms could produce hydrocarbons on a commercial scale

- The successful utilization of hydrocarbon production by MOS may depend on their genetic modification and selection of efficient hydrocarbon-producing strains.

- Studies have shown that a ten fold increase in hydrocarbon production can be achieved with *Micrococcus* through genetic transformation.

- Theoretically, the algae and cyanobacteria that produce hydrocarbons may permit the **conversion of solar energy to chemical forms of energy** that could be utilized to power conventional combustion engines.

Hydrogen

- Hydrogen is an ideal fuel as on combustion the only product is water
- This makes it a very clean fuel which adds no other greenhouse gases to the environment
- Hydrogen can be used as an automotive fuel as well as a fuel for the generation of electricity
- Hydrogen, not presently a major fuel, can be produced by various microorganisms and could become an important fuel resource

- Hydrogen is produced as an **end product from the fermentation of carbohydrates** by bacteria that carry out a mixed acid fermentation.
  - an anaerobic fermentation where the products are a complex mixture of acids, particularly lactate, acetate, succinate and formate as well as ethanol and equal amounts of H_2 and CO_2. It is characteristic for members of the Enterobacteriaceae family

- Rapid hydrogen removal favors the fermentation process.

- The theoretical efficiency of the conversion of carbohydrates to energy stored in hydrogen is approximately 33%, compared to 85% for methane formation from organic matter.

- Methane formation from organic compounds, rather than hydrogen formation, should therefore be considered as the preferred process for microbial energy production via fermentation.

Thermal Processes

One type of thermal process uses the energy stored in such resources as coal or biomass to simply release the hydrogen contained within their molecular structures. Another type uses heat in combination with closed chemical cycles to produce hydrogen from feedstocks, such as water; these are known as "thermochemical" processes.

- Distributed Natural Gas Reforming
- Bio-Derived Liquids Reforming
- Coal and Biomass Gasification
- Thermochemical Production
  (Using a Heat-Driven Chemical Reaction to Split Water)

Electrolytic Processes

Water electrolysis uses electricity to split water into hydrogen and oxygen. Hydrogen produced via electrolysis can result in zero greenhouse gas emissions, depending on the source of the electricity used.

- Water Electrolysis
  (Splitting Water Using Electricity)

Photolytic Processes

Photolytic processes use light energy to split water into hydrogen and oxygen. Currently in the very early stages of research, these processes offer long-term potential for sustainable hydrogen production with low environmental impact.

- Photoelectrochemical Hydrogen Production
  (Using Solar Power to Directly Split Water)
- Biological[1] Hydrogen Production
  (Photobiological Water Splitting)

- Various microorganisms are capable of **biophotolysis**, that is, the production of hydrogen from water, using solar energy
- Hydrogen production occurs in various photosynthetic bacteria, including **purple and green sulfur bacteria**, and in some **cyanobacteria**
- The photosystems of cyanobacteria, algae, and plants are all capable of splitting water, releasing free oxygen and using the hydrogen to reduce carbon dioxide to carbohydrate

- Various microorganisms are capable of **biophotolysis**, that is, the production of hydrogen from water, using solar energy

- Hydrogen production occurs in various photosynthetic bacteria, including **purple and green sulfur bacteria**, and in some **cyanobacteria**

- The photosystems of cyanobacteria, algae, and plants are all capable of splitting water, releasing free oxygen and using the hydrogen to reduce carbon dioxide to carbohydrate

Hydrogen production

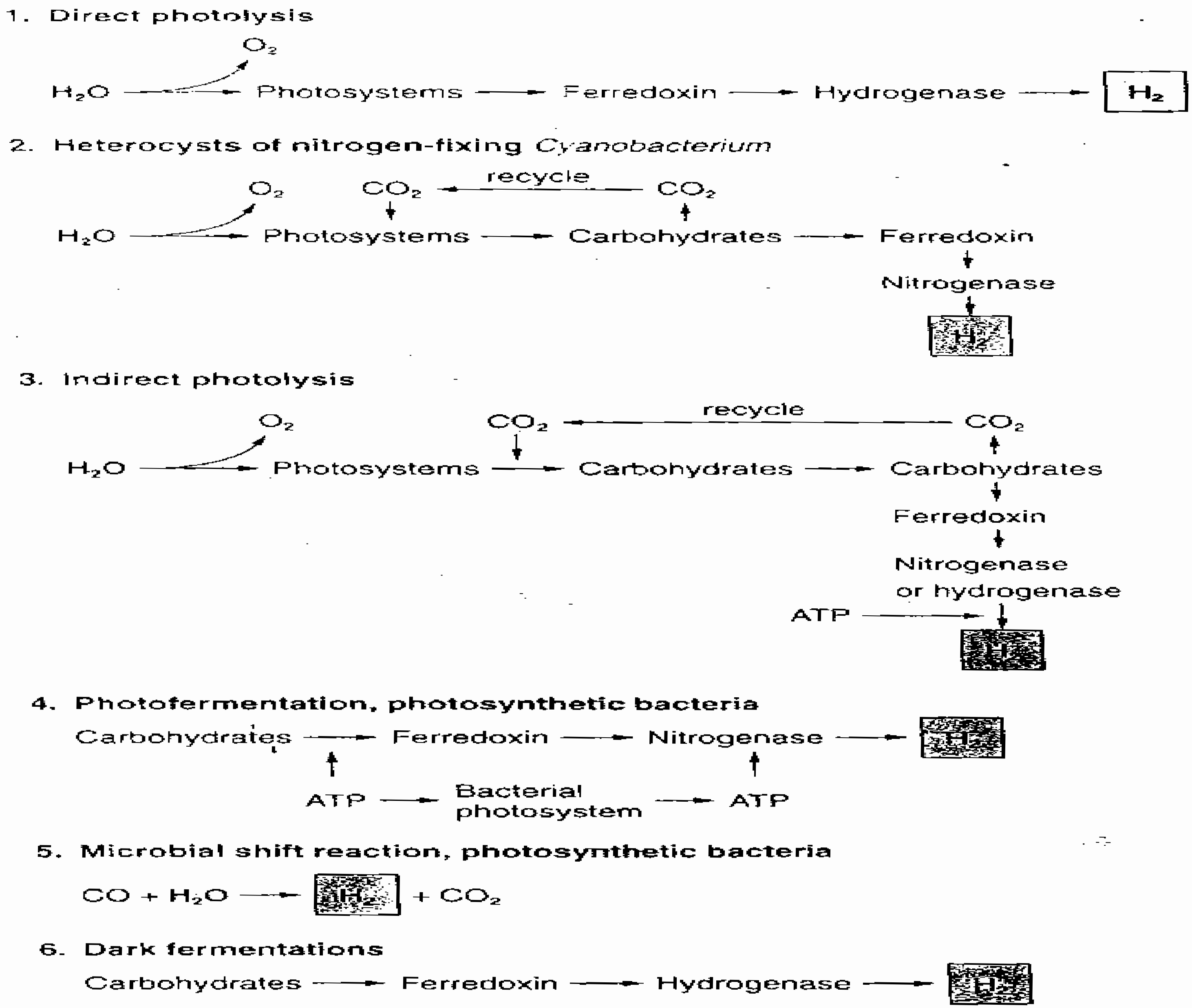


Fig. 6.16 The possible processes for the biological production of hydrogen (from Benemann, 1996).

- Photoproduction of hydrogen by biological systems is still far from the stage of practical utilization.
- Various environmental conditions affect the rates of evolution of hydrogen gas through biophotolysis.
- In some nitrogen-fixing cyanobacteria, it is possible to release hydrogen effectively through biophotolysis when the organisms are nitrogen starved.
- Photoheterotrophic bacteria, such as *Rhodospirillum* or *Rhodopseudomonas,* can be used for hydrogen production from waste materials.

Future developments

- A lack of knowledge about many areas of biophotolysis prevents assessment of the feasibility of producing and utilizing hydrogen generated by microorganisms on a large scale
- The prospects for developing and employing biophotolysis as a viable future energy source, therefore, are uncertain
- It can be expected, however, that efforts will be made in the future to determine the feasibility of utilizing photosynthetic microorganisms in the conversion of solar energy to hydrogen gas

New Technologies

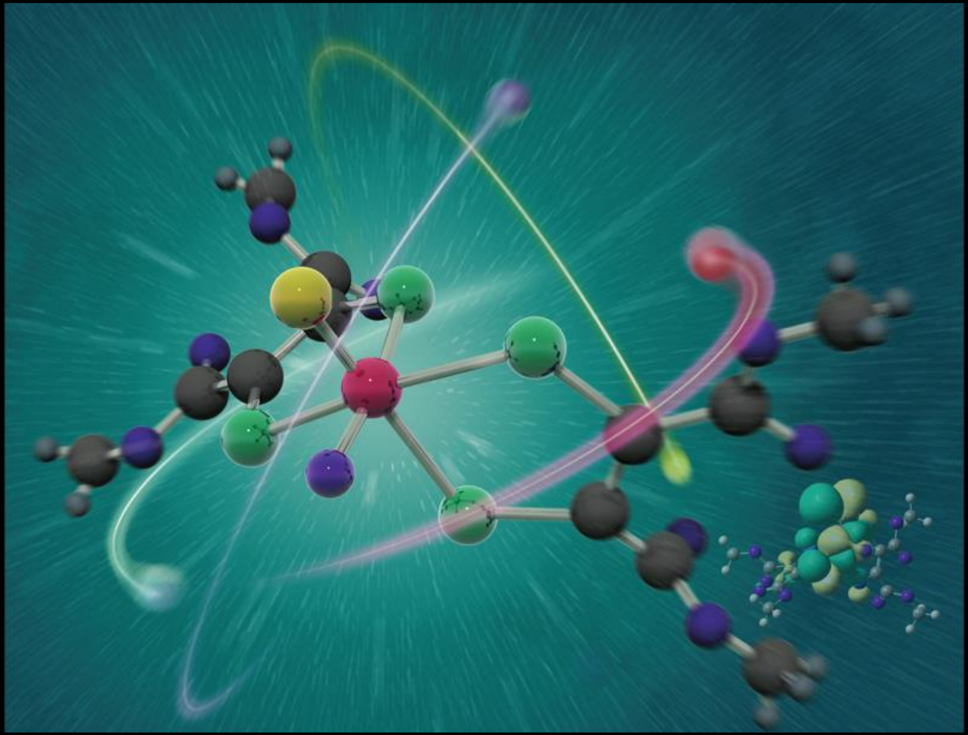
- Hydrogen Production from H_2O:

http://cleantechnica.com/2013/08/02/radically-new-technique-to-produce-hydrogen-fuel-from-water-developed/

- An enormous solar-thermal system.
- Sunlight is concentrated via array of mirrors.
- Heats tower to 2500 °F.
- Heat reduces metal oxides to release O_2.
- Produces unstable metal atoms wanting O atoms.
- Introduce steam from H_2O.
- O is captured by unstable metal, therefore releasing H_2.

New Technologies

- Hydrogen Production from use of catalyst:

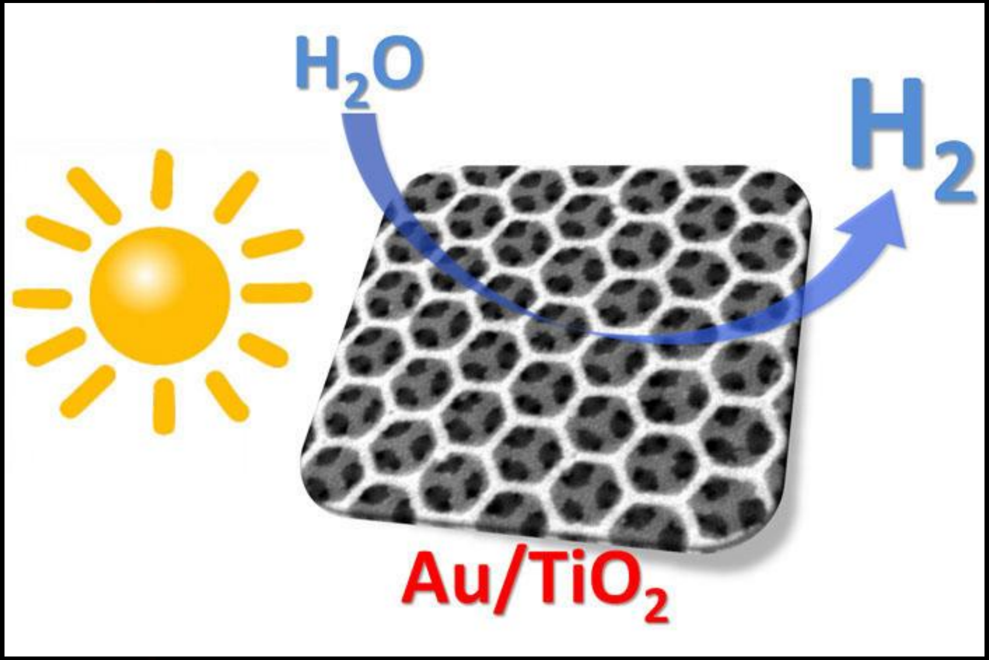


http://phys.org/news/2013-08-catalyst-hydrogen.html

- Few catalysts are energy efficient, highly active, stable and react with H_2O.
- New nickel-based catalyses reaction with H_2O to produce H_2.
- Produces 170,000 H per second!

New Technologies

- Hydrogen Production from use of catalyst & sunlight:



http://www.sciencedaily.com/releases/2013/12/131213093310.htm

- Harnesses the properties of photonic crystals and nanoparticles of metal.
- Produces more H_2 than other technologies.
- Photonic crystals captures light to excite the metal electrons to capture oxygen from water to release H_2.
- This process occurs at room temperature!

New Technologies

- Hydrogen Production from charcoal powder & lasers

http://cleantechnica.com/2013/08/31/inexpensive-hydrogen-fuel-from-water-producing-hydrogen-with-charcoal-powder-and-lasers/

- Very cheap alternative!
- Added carbon and charcoal powders to H_2O and a laser beam at nanopulses to the mixture.
- H_2 was generated at room temperature without costly catalysts or electrodes.

Conclusions

- The need to reduce green house gases originating from fossil fuels
- The world's oil supply is gradually running out
- The world's demand for liquid transport fuels can triple in the next fifty years

- Microbiology can therefore make a significant contribution to solving the world energy problems.