Practical environmental biotechnology

Lecture 02

Industrial biotechnology

Exam

Reports

Exam: written, short answer questions

21.06, 10:00, room 1.1.3

Biomining: bioleaching

Extraction of specific metals from their ores through the use of microorganisms.

Based on the ability of micro-organism (bacteria and fungi) to transform solid compounds in soluble and extractable elements, which can be recovered.

Represents a ‘clean technology’ process with low cost and less energy consumption as compared to conventional methods.

Metals for which this technique is mainly employed include: copper, cobalt, nickel, zinc, uranium, gold, silver.

Extraction either from insoluble sulfides or (uranium) from oxides.
Recovery of gold and silver: removing interfering metal sulfides from ores prior to cyanidation treatment.

History

Microorganisms have contributed to the solubilisation of metal sulphides since ancient times – Mediterrean region, 3000 years ago

Copper mine of Rio Tinto in Spain (300 years ago) was probably the first large-scale operation in which microorganism played a major role.

1947, 1950 – the role of bacteria was shown, discovery of specific species

In the period of 1950 to 1980, bioleaching was thought mostly as an appropriate technology for the recovery of copper and other metals from damps and low-grade ores.

Application of microbial processes by the mineral industry, biohydrometallurgy, predates the understanding of the role of microorganisms in metals extraction.

Biochemistry

"Direct mechanism": the direct enzymatic oxidation of the sulfur moiety of heavy metal sulfides?

„Indirect mechanism": non-enzymatic metal sulfide oxidation by Fe(III) ferric ions combined with enzymatic (re) oxidation of the resulting Fe(II) ferrous ions comprises two submechanisms: "contact" and "non-contact" mechanisms.

Non-contact mechanism: bacteria oxidize Fe(II) ions in solution to Fe(III) ions, which somehow come into contact with a mineral surface, where they are reduced, and enter the cycle again.

The contact mechanism takes into account that most cells attach to the surface of sulfide minerals. The electrochemical processes resulting in the dissolution of sulfide minerals take place at the interface between the bacterial cell (wall) and the mineral sulfide surface.

In both contact and non-contact mechanisms, the bacteria contribute to mineral dissolution by generation of the oxidizing agent, the Fe(III) ion, and by subsequent oxidation of the sulfur compounds resulting from the dissolution.

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Organisms involved

Metabolism	Species	Leaching factor	pH	Temp. [°C]	Taxon
Chemo-lito-autotrophy	*Ferroplasma acidophilum*	Fe^{3+}	1.3-2.2	15-45	Archea
	Sulfolobus	Fe^{3+} Sulphuric acid	1.0-6.0	85, thermophilic	
	Leptospirillum ferrooxidans	Fe^{3+}	2.5-3.0	30	Bakteria
	Leptospirillum thermoferrooxidans	Fe^{3+}	1.7-1.9	45-50	
	Acidithiobacillus ferrooxidans	Fe^{3+} Sulphuric acid	1.4-6.0	28-35	
	Acidithiobacillus thiooxidans	Sulphuric acid	0.5-6.0	10-37	
Hetero-trophy	*Bacillus megaterium*	Citrate			
	Metallogenium sp.	Fe^{3+}	3.5-6.8		

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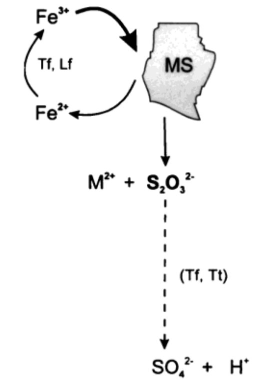
Thiosulfate pathway



FeS_2 pyrite, MoS_2 molybdenite, WS_2 tungstenite

Tf – *Acidithiobacillus ferrooxidans*

Tt - *Acidithiobacillus thiooxidans*

Lf - *Leptospirillum ferrooxidans*

$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+$

$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+$

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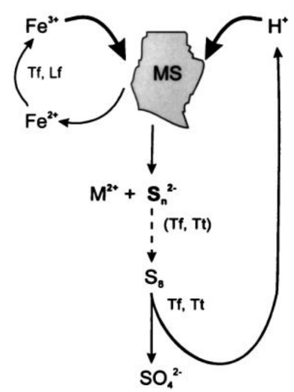
Polysulfide pathway



PbS sphalerite, $CuFeS_2$ galena,

ZnS arsenopyrite, MnS_2

chalcopyrite, As_2S_3 chalcopyrite,

As_3S_4 hauerite

Tf – *Acidithiobacillus ferrooxidans*

Tt - *Acidithiobacillus thiooxidans*

Lf - *Leptospirillum ferrooxidans*

$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0.5H_2Sn + Fe^{2+}$

$0.5H_2Sn + Fe^{3+} \rightarrow 0.125S_8 + Fe^{2+} + 2H^+$

$0.125S_8 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$

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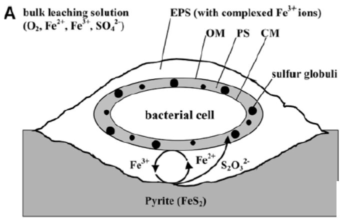
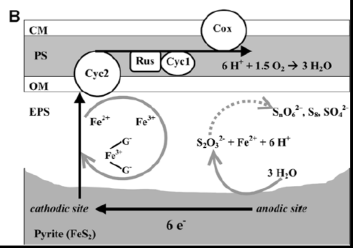
Contact leaching mechanism: *Acidithiobacillus ferrooxidans*



Bacterial cell embedded in its EPS attached to pyrite via electrostatic interactions.

EPS- extracellular polymeric substance
CM - Cytoplasmic membrane,
PS - periplasmic space,
OM – outer membrane.



Detail of the electron transport from pyrite to molecular oxygen (black arrows).

G - glucuronic acid residues
Cyc2 – cytochrome
Cox - cytochrome oxidase
Rus - rusticyanin

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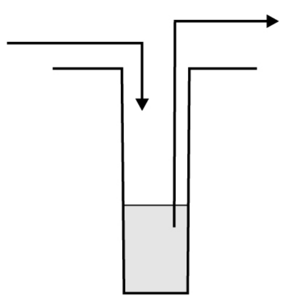
Extraction of metals

In situ extraction



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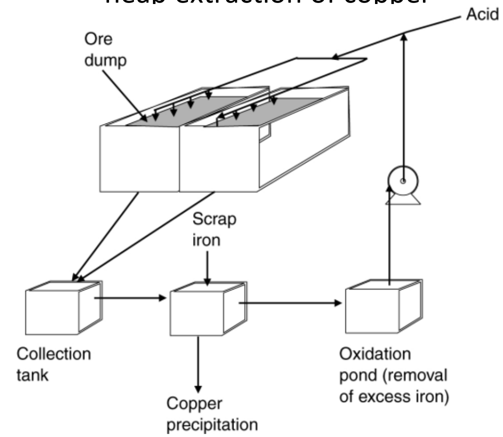
Ex situ – heap extraction of copper



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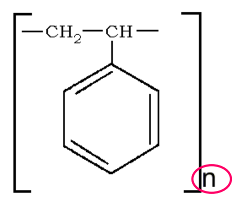
Traditional plastics

$\left[-CH_2-CH_2-\right]_n$ Polyethylene



Polystyrene

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Problem of plastics

Solutions

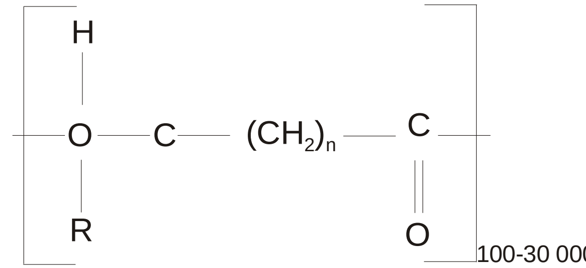
Photodegradable or semi-biodegradable or 100% biodegradable:

1. modification of existing material,
2. chemical co-polymerisation of known biodegradable material,
3. use of biopolymers for making plastics.

Biodegradable plastic materials under development are:

1. PHAs;
2. Polylactides
3. Aliphatic polyesters
4. Polysaccharides
5. Co-polymers and/or blends of above.

PHA structure



- Polyesters of hydroxy acids.
- C.a. 100 different monomers.
- PHB (poly-β-hydroxybutyrate) – most common.

Properties of PHB

1. Water insoluble and relatively resistant to hydrolytic degradation. This differentiates PHB from most other currently available biodegradable plastics, which are either water soluble or moisture sensitive.
2. Shows good oxygen permeability.
3. Has good ultra-violet resistance but has poor resistance to acids and bases.
4. Is soluble in chloroform and other chlorinated hydrocarbons.
5. Is biocompatible and hence is suitable for medical applications.
6. Has melting point 1750C., and glass transition temperature 150C.
7. Has tensile strength 40 MPa which is close to that of polypropylene.
8. Sinks in water while polypropylene floats, but its PHB facilitates anaerobic biodegradation in sediments.
9. PHB is nontoxic.

History

1923, Lemoigne, Institut Pasteur: aerobic spore-forming bacillus, formed quantities of 3-hydroxybutyric acid in anaerobic suspensions.
1927, extractration of a substance from Bacillus using chloroform and prove that the material was a polymer of 3-hydroxybutyric acid.

Until 1960s the production of poly(3-hydroxybutyrate), P(3HB), was not explored on a commercial scale.

The oil crisis of the 1970s provided a boost in the quest for alternative plastics.

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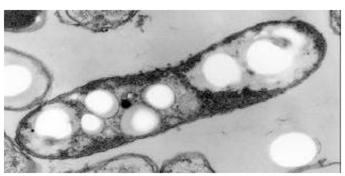
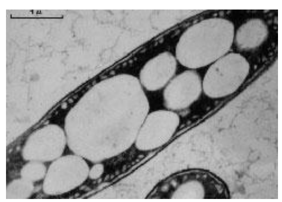
PHB in bacteria



Rubrivivax gelatinosus



Alcaligenes latus

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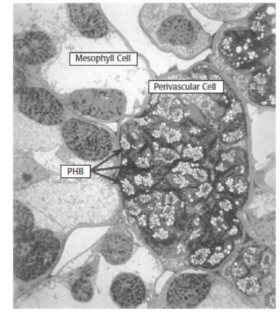
PHB in plants



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PHB accumulation in Prokaryote

Organism	% dry weight
Ralstonia eutropha (*Alcaligenes eutrophus*)	96
Azospirillum	75
Azotobacter	73
Beggiatoa	57
Leptothrix	67
Methylocystis	70
Pseudomonas	67
Rhizobium	57
Rhodobacter	80

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Biosynthesis

List of limiting components leading to PHA formation:

- Ammonia - *Alcaligenes eutrophus*, also others
- Carbon - *Spirillum* spp., *Hypomicrobium* spp.
- Iron, Mg - *Pseudomonas* spp.
- Mn, O_2 - *Azospirillum*, *Rhodobacter* spp.
- PO_4 - *Rhodospirillum*, *Rhodobacter* spp.
- Potassium sulfate *Bacillus*, *Rhodospirillum*, *Rhodobacter* etc.

Enzymes PHA biosynthesis: PHA synthase, beta ketothiolase and reductase. PHA depolymerase: degrades the polymer and uses the breakdown metabolites for cell growth.

Groups of PHAs

The number of constituent carbon atoms in their monomer units:
– short-chainlength (SCL), monomers with 3–5 carbon atoms, are stiff and brittle with a high degree of crystallinity

– medium-chain-length (MCL) monomers with 6–14 carbon atoms, flexible, have low crystallinity, tensile strength and melting point.

Organisms applied in PHB production

Ralstonia eutropha, *Spirillum* spp., *Hypomicrobium* spp., *Pseudomonas* spp., *Azospirillum*, *Rhodobacter* spp., *Rhodospirillum*.

Transgenic bacteria: *Escherichia coli*, *R. eutropha.*

Transgenic plants: *Arabidopsis thaliana*

Cyanobacteria: *Synechococcus sp.*

Application of PHAs

- Packaging films (for food packages), bags, containers, paper coatings.
- Biodegradable carrier for long term dosage of drugs, medicines, insecticides, herbicides, insecticides or fertilizers.
- Disposable items such as razors, utensils, diapers, feminine hygiene products, cosmetics containers, shampoo bottles, cups etc.
- Medical applications - Surgical pins, sutures, staples, swabs, wound dressings, bone replacements & plates and blood vessel replacements, Stimulation of bone growth by piezoelectric properties.

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Comparison of PHB with poliethylene

Polimer	Production CO_2 kg^{-1}	Energy demand MJ kg^{-1}
PHB	2.6	44.7
Polyethylene	3.0	81.8

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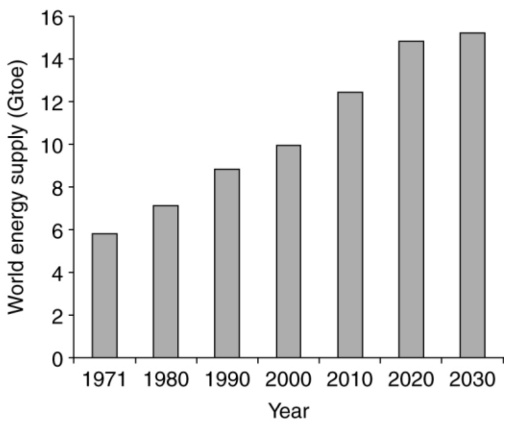
Increase in the supply of the energy to the world



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Crude oil consumption

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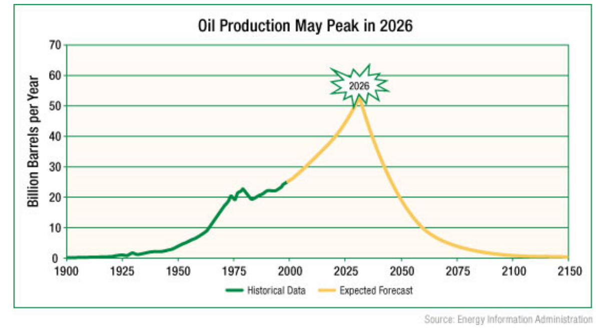

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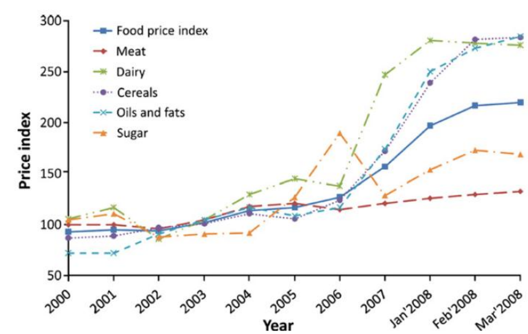
Increase of food price



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Biofuels– area of cultivation

15% needs of transport in USA

Crop	Oil production (l/ha)	Area (mln ha)
Maize	172	462
Soya bean	446	178
Rape bean	1,190	67
Jatropha	1,892	42
Palm oil	5,950	13
Algae/cyanobacteria (30% of oil)	59,000	1.3
Algae/cyanobacteria (70% of oil)	137,000	0.6

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Biofuels

1. First generation ‘‘rediscovered’’ biofuels; ethanol and butanol (*Clostridium acetobutylicum*) from sugar and grains, and diesel from oilseeds.

2. The second generation

Cropping solutions. Lignocellulosic materials as a substrate: straws, specially cultivated grasses: ‘‘switchcanthus’’ (switchgrass, *Panicum virgatum*, *Miscanthus*).

Fermentation of pentoses.

Biodiesels from: castor bean (*Ricinus communis* L.), *Pongamia pinnata, (Calophyllum L. C. inophyllum),* Jatropha *(Jatropha curcas).*

3. Third generation technologies: algae and cyanobacteria for biofuel production

4. Fourth generation technologies — biohydrogen

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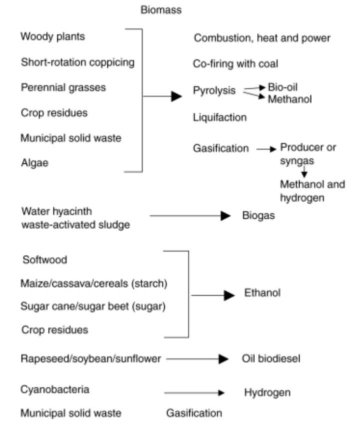
Possible sources of energy from bological materials



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Ethanol

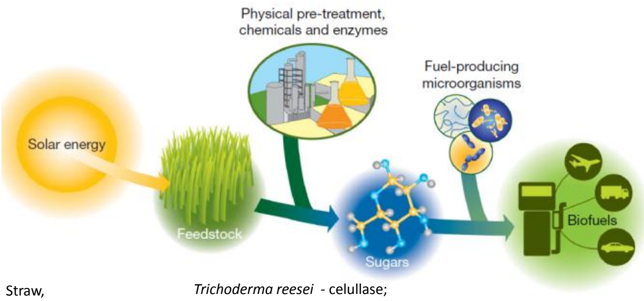
1. Sugar (sugar cane, sugar-beet); *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* (tolerate high osmotic pressure)

2. Starch (*Bacillus licheniformis, Escherichia coli Bacillus subtilis*); maize, wheat, cassava

3. Buthanol: *Clostridium acetobutylicum*, starch

4. Fermentation of agricultural wastes (e.g. bagassa –sugar cane)

Degradation of biomass



Straw,
Panicum virgatum
Miscanthus

Trichoderma reesei - celullase; cellobiohydrolase, endoglucanase, glucosidases, hemicellulase

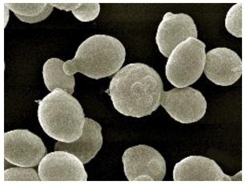
Organic wastes - lignin

Phanerochaete chrysosporium, "white-rot" fungi, degrades lignin

Pentose degradation

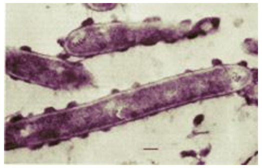
Pichia stipitis,

Candida shehatae, Pachysolen tannophilus.



Clostridium thermocellum,

C. thermohydrosulfuricum, Thermoanaerobacter thermosaccharolyticum.



Biodiesel

Jatropha curcas

Ricinus communis L.

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Biodiesel

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Calophyllum inophyllum L.

Pongamia pinnata

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Biodiesel – algae

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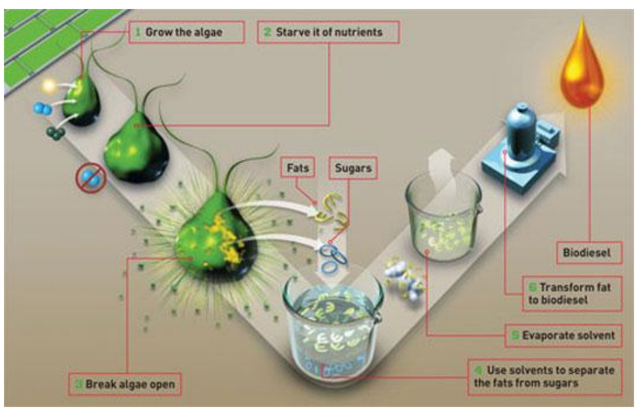

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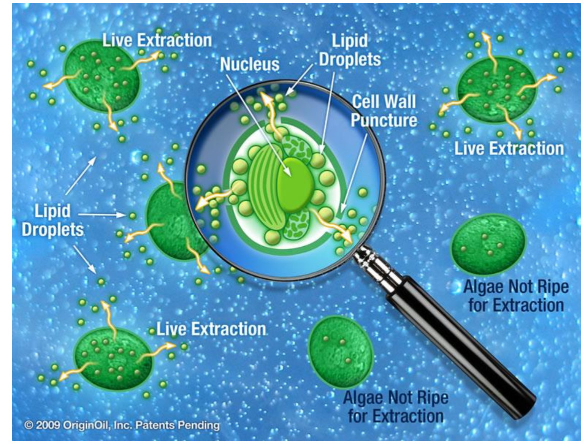

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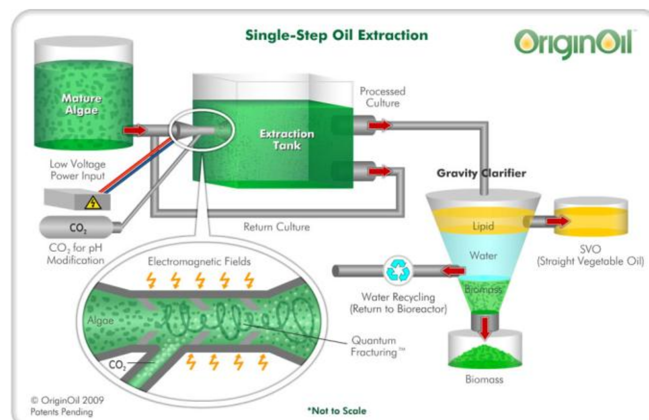

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Biodiesel – algae

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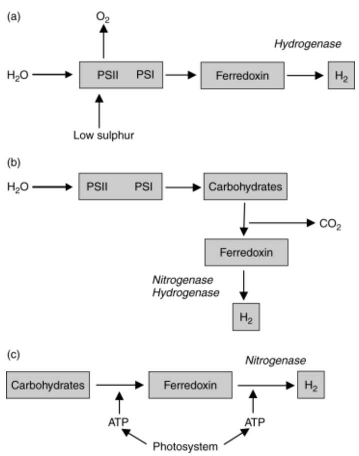
Bioprocess for hydrogen generation



Biophotolysis

Green algae: *Chlamydomonas reinhardtii, Chlorella fusca, Scenedesmus obliquus, Chlorococcum littorale, Platymonas subcordiformis*

Photofermentation

Purple non-sulfur phototrophic bacteria: *Rhodospirillum rubrum, Rhodopseudomonas palustris, Rhodobacter sphaeroides*

Rhodobacter capsulatus

Dark fermentation

Clostridium spp, Pseudomonas spp, Vibrio spp, Actinomyces spp

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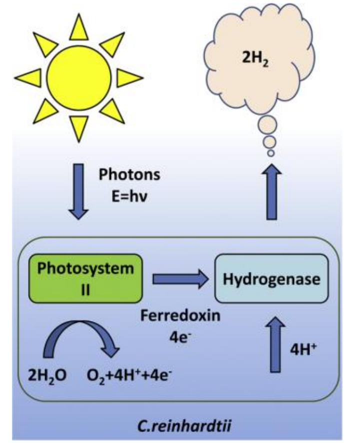
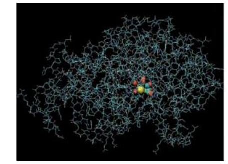
Biophotolysis: *Chlamydomonas reinhardtii*





Hydrogenase

Tamburic i in. 2010

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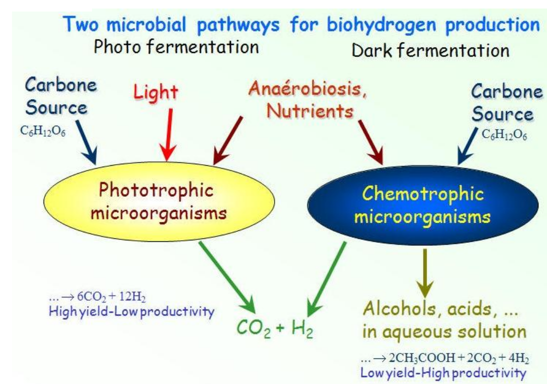
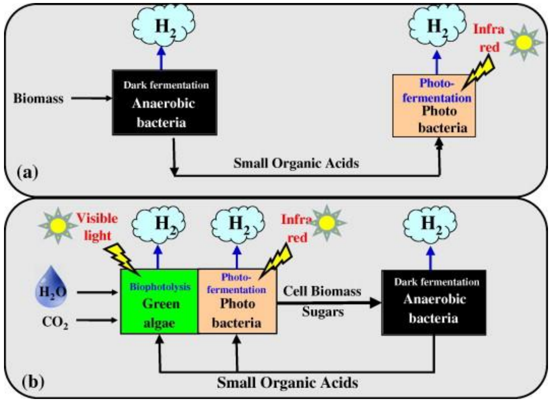
Biohydrogen - fermentation



www.microh2.ulg.ac.be/PROJECT1.html

Biohydrogen - production



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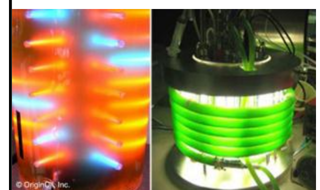
Biohydrogen



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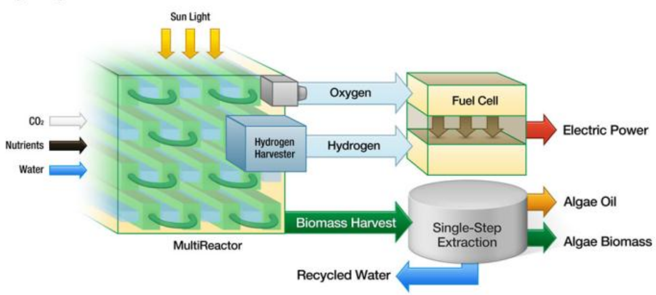
Hydrogen Harvester



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Biogas

Kitchen garbage, Sewage sludge, Livestock waste, Wastewater
Polysaccharides (cellulose), Proteins, Fats

Fermentative bacteria ⇩

Sugars, Amino acids, Fatty acids, Glycerol

Acetogenic bacteria ⇩

Acetate, Hydrogen+Carbon dioxide

Methanogens ⇩ Anaerobic conditions

Methane+Carbon dioxide

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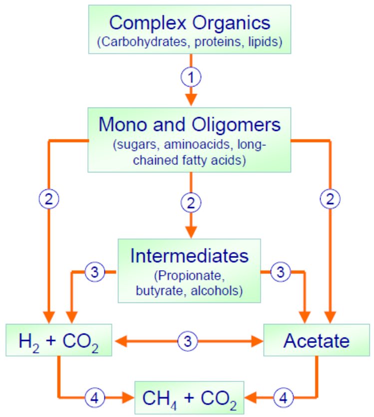
Anaerobic digestion



1. Hydrolysis
– Long chain polymers broken down to smaller molecules

2. Acidogenesis
– Production of hydrogen and volatile fatty acids (VFAs)

3. Acetogenesis
– alcohols, VFAs converted to acetate and hydrogen

4. Methanogenesis
– Hydrogen and acetate converted to methane

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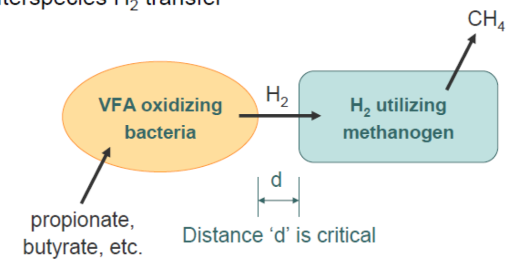
Role of Interspecies Hydrogen Transfer

Interspecies H_2 transfer



VFAs - volatile fatty acids

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Methanogens

- Belong to the Archaea.
- Are strict anaerobes they evolved billions (3.5 billion) years ago when the earth had very low or zero levels of oxygen.
- Utilize simple inorganic substrates such as H_2, CO_2 or simple organic substrates such as acetate, formate and methanol

Types of methanogenesis
Hydrogen (Lithotrophic methanogens): $4H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$
(ca 20% of methane)
AND/OR
Acetate (acetoclastic methanogens): $CH_3COOH + H_2O \rightarrow CH_4 + CO2$
(ca 80% of methane)

Examples:
Genus: *Methanosarcina (*acetotrophic, litotrophic*), Methanosaeta (*acetotrophic*)*

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Energetic value of biogas

Biogas – 50-70% of methane

1 m^3 equals:

- 0,5 m^3 of natural gas;
- 0,7 l of diesel fuel;
- 0,7 l of gasoline;
- 0,8 kg coke (fuel, coal);
- 1,2 kg black coal;
- 2,2 kg wood.

Figure 5: Small "foil-plant" (Ivory Coast)