Bioraffinerie in EU e Italia

Waste as a solution?

The **1.3 x 10^9 t** of food waste generated worldwide per annum constitute a renewable resource for chemical production that at least in terms of capacity should not be neglected. However, direct comparison is misleading because the carbon content of food waste streams is lower due to the high water content of many food waste streams.

For instance, despite the high quantities of whey produced annually from the dairy industry, the high water content (more than 90%) requires pretreatment steps including ultrafiltration membranes to separate protein isolates and evaporation to concentrate the remaining lactose that could be used as carbon source in fermentation processes.

Food wastes are generated through the whole food supply chain in the following three major stages: (1) production and storage of raw material (mainly in third world countries), (2) industrial processing, and (3) municipal waste disposal including domestic consumption and out-of-date or unconsumed products disposed from restaurants, supermarkets and various catering services. In recent years, food waste valorisation is gaining momentum and could play a pivotal role in the bio-economy era regarding chemical and biopolymer production. Recent focus on the development of advanced biorefining concepts for the production of chemicals, materials and fuels from food supply chain waste.

Valorisation of municipal food waste streams via fermentation needs different processing strategies, such as logistics, collection, fractionation based on type of waste, quality issues, high heterogeneity and need for construction of new plants among others, compared to industrial food waste streams where integration of new processing lines in existing industrial facilities is feasible.

Scarti agroalimentari

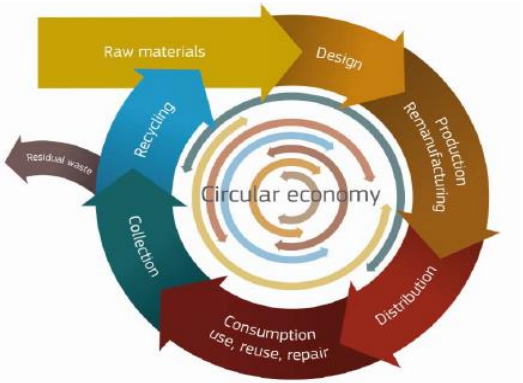
- Prodotti partendo dall'ambito agricolo di trasformazione industriale fino al contesto domestico
- Circa 1,3 miliardi di tonnellate di rifiuti alimentari mondiali annuale
- Conseguenze sulla sostenibilità ambientale, lo spreco delle risorse e la salute umana
- Serbatoio di sostanze chimiche quali : proteine, carboidrati, lipidi e acqua

Settore alimentare	produzione processi	% di scarti e sottoprodotti
settore ittico	Conseve di pesce	30-65
	Sfilettatura del pesce, stagionamento, salatura e il fumo	50-75
	Elaborazione crostacei	50-60
	Trattamento molluschi	20-50
settore di carne	macellazione carne Bovina	40-52
	macellazione del maiale	35
	macellazione del pollame	31-38
settore lattiero-caseario	Il latte, il Burro e la produzione di crema	trascurabile
	La produzione di Yogurt	(2-6)
	formaggio fresco morbido e cotto	85-90
settore vitivinicolo	Produzione di vino bianco	20-30
	produzione di vino rosso	20-30
settore ortofrutticolo	produzione di frutta e succhi vegetali	
	Tasformazione e conservazione di frutta e verdura	(5-30)
	Produzione di Olio vegetali	40-70
	La produzione di amido di Mais	80
	la produzione di amido di frumento	50

Scarti agroalimentari

Alcune esempio tipo di scarti alimentarri	contenuto di acqua (%)	contenuto carboidrati (%)	contenuto proteina (%)	contenuto olio/grasso
scarti di carne	41		24.6	69.9
scarti di pesce	73.9	_	57	19.1
farina di soia	10	29.9	42	4
farina di seme di lino	8	38	36	0.5
lievito di birra da birrerie	5	39.5	43	1.5
lievito idrolizzato	5.5	_	52.5	_
farina di pesce	8		65	3.8
Pharmamedia (derivato dall'embrio del cottone)	1	24.1	59.2	4
siero	92.7	4.9	0.9	0.9
crusca del frumento	11	64.5	15.5	4.2
polpa di pomodoro (base secca)		25.4-50 (fibra)	15.4-23.7	5.4-20.5

- Materie prime utilizzabili nell'ottica dell'economia circolare



Siero di latte

- Sottoprodotto della produzione del formaggio
- Produzione mondiale stimata a 180-190 milioni di tonnellate l'anno
- Inquinante ambientale (BOD= 27- 60g/L)
- Componenti proteici del siero:

proteine	concentrazioni (g/L)	peso molecolare (Kda)	numeri di amminoacidi
β-lattoglobulina	1.3	18,277	162
α-lattoalbumina	1.2	14,175	123
sieroalbumina	0.4	66.267	582
Immunoglobuline	0.7	25,000 (catena leggera) + 50,000 (catena pesante)	_
Lattoferrina	0.1	80,000	700
Lattoperossidasi	0.03	70,000	612
Glicomacropeptide	1.2	6700	64

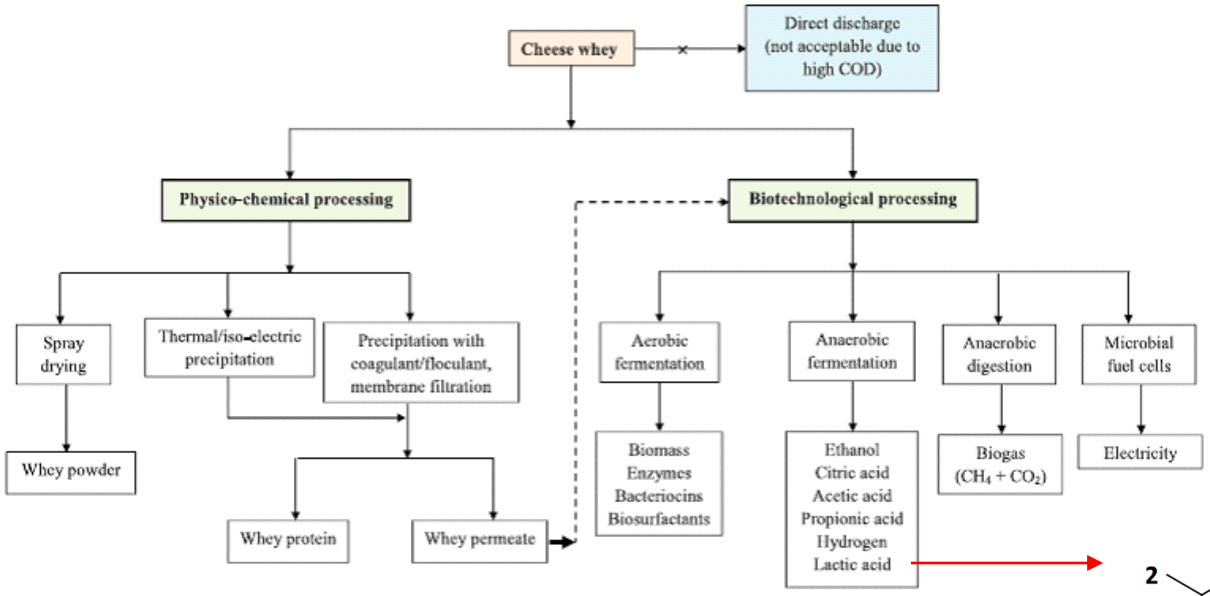
Esempi di applicazioni:

- Idrolizzati per formulati per l'infanzia
- Integratori alimentari
- Peptidi per la cosmetica

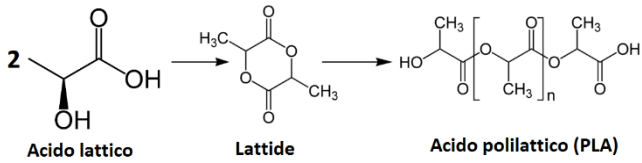
- Integratori per pazienti sofferenti di fenilchetonuria

Siero di latte

- Valorizzazione del siero del latte come:
- Concentrati di proteine (WPC)
- Isolati di proteine (WPI)
-



Trasformazione del siero di latte con metodi chimico-fisici e biotecnologici (Singh et al. 2015)



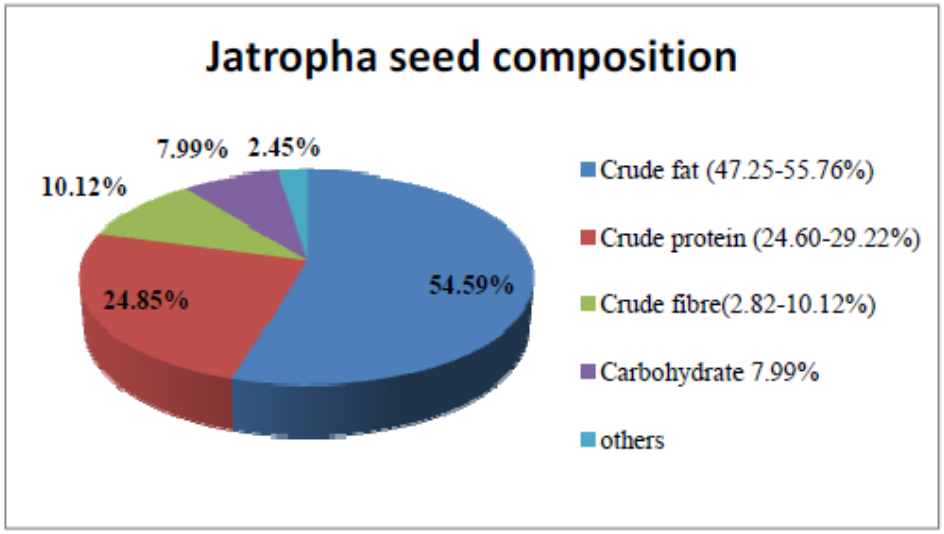
Acido lattico — Lattide — Acido polilattico (PLA)

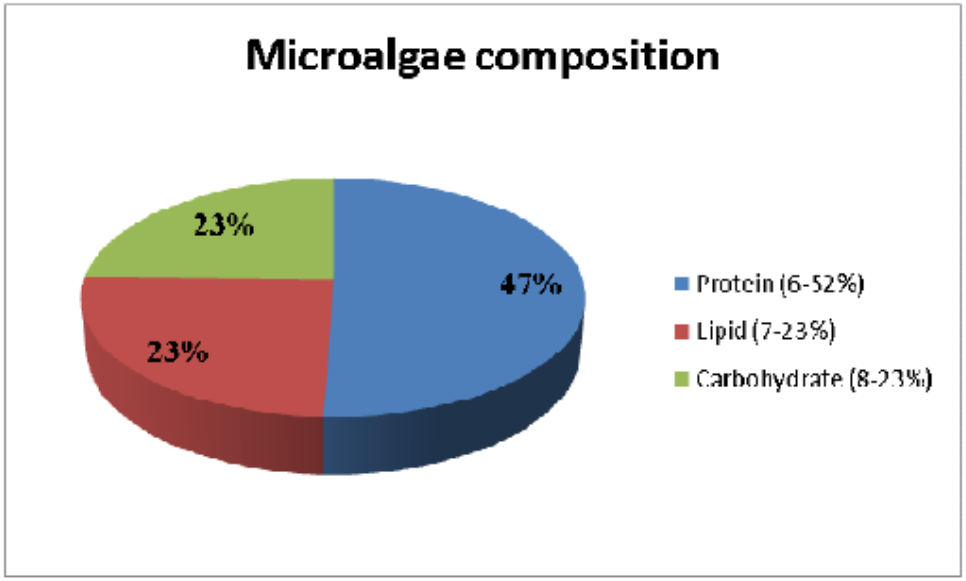
Esempi di biomolecole estratte con l'ausilio di enzimi

Product type	Product	Source	Enzyme used	Maximum yield (%)
Oils and carotenoids	Oil	Grape seed	Cellulase, protease, xylase and pectinase	17.5
	Carotenoids	Marigold flower	Viscozyme, Pectinex, neutrase, corolase and HT-proteolytic	97
	Volatile oil	Mandarin peel	Xylan-degrading enzymes	15
	Carotene	Carrot pomace	Pectinex Ultra SP-L	0.0064
	Lycopene	Tomato	Pancreatin	2.5-fold
		Tomato	Cellulase and pectinase	206
	Capsaicin	Chilli	Cellulase, hemicellulase and pectinase	n.d.[a]
	Colourant	Pitaya	Pectinolytic, hemicellulolytic and cellulolytic enzymes	83.5
	Anthocyanin	Grape skin	Pectinex BE3-L	n.d.[a]
Glycosides	Sugar	Grapefruit peel waste	Cellulase and pectinase	0.6377
	Oligosaccharide	Rice bran	Cellulase	39.9
	Inulin	Jerusalem artichoke	Inulinase	n.d.[a]
	Starch	Cassava	Pectinase enzyme	45.6
	Pectin	Pumpkin	Xylase, cellulose, β-glucosidase, endopolygalacturonase and pectinesterase	14.0
Others	Vanillin	Vanilla green pods	β-glucosidase and pectinase	14–21
	Flavonoid (naringin)	Kinnow peel	Recombinant rhamnosidase	n.d.[a]
	Phenolics	Citrus peel	Celluzyme MX	65.5
	Proteins	Lentils and white beans	Glucoamylases	50.3
	Polyphenols	Grape pomace	Pectinolytic	98.1
	Catechins	Tea beverage	Pepsin	80
	Lignans	Flax	Cellulase and glycosidase	40.75 mg/g
	Soluble fibre	Carrot pomace	Cellulase-rich crude preparation	77.3

[a]Abbreviation: n.d., not defined.

Non-food sources of oils





Biodiesel from recycled oils: the case of Graz

- From 1990 employs 40 tracks using 10-15% **RFO-ME** (**R**ecycled-**F**riying **O**il **M**ethyl **E**ster) corresponding to 1.000.000 L / year

http://www.frikus.com

Graz: program “From the frying pan into the tank”: 250 restaurant and privates collecting frying oils used for producing biodiesel for public transportation (135 buses)

8.5 million Km/year

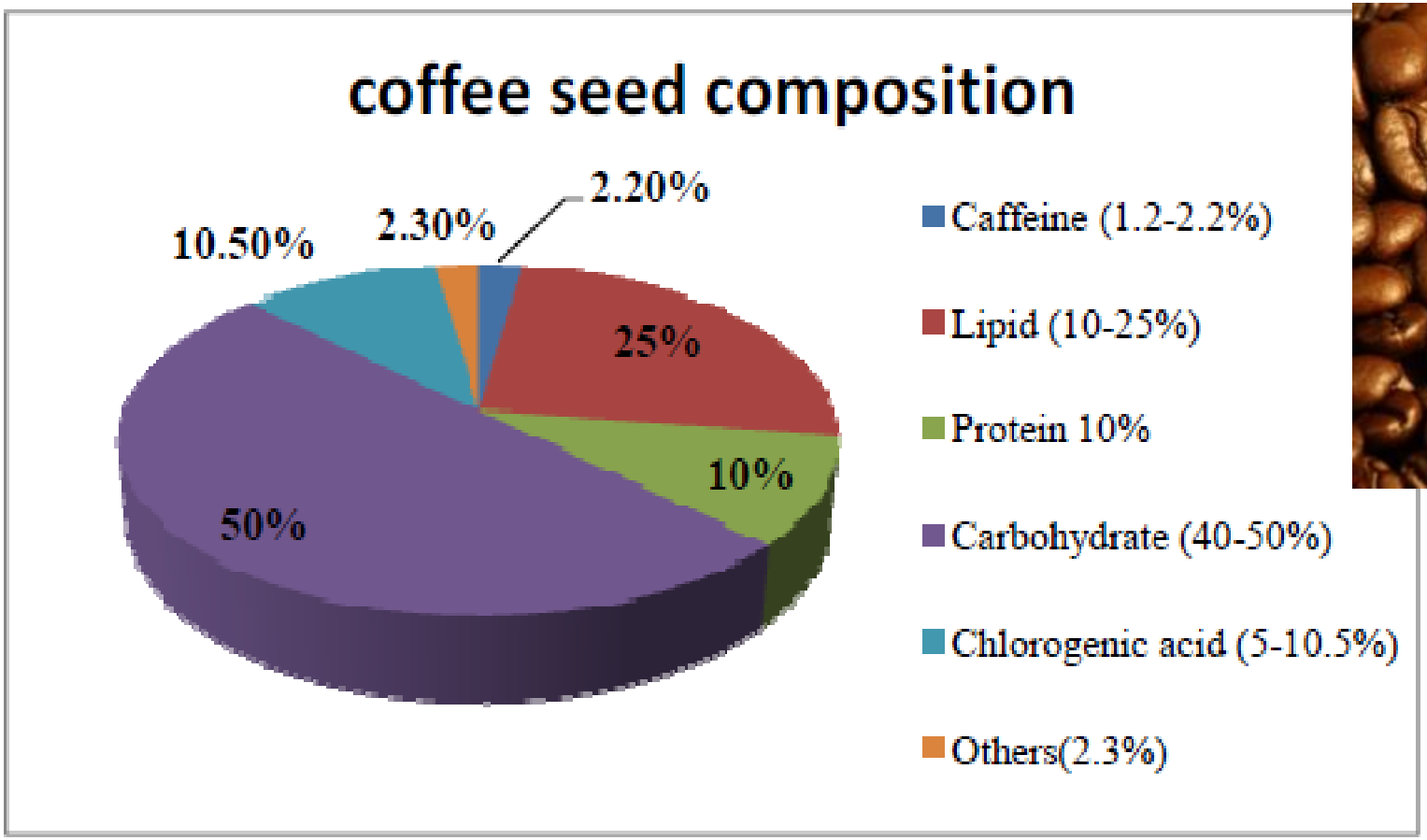
3.8 million L/year

www.oekoservice.at

«Circular chemistry»: waste as a solution?

1.3 x 10^9 t of food waste generated worldwide per annum

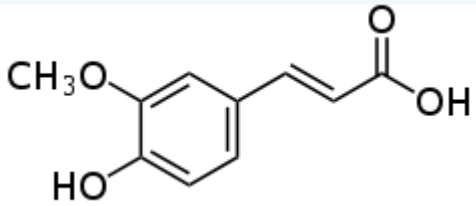
- World coffe production: 5.9 million tons (first raw material exported by size after crude oil)
- Italy: 324.000 ton



Lipids:
Triglycerides 75%
Esters of diterpenes or fatty ac. 18%

Tryptamine derivatives, sterols,…

Ferulic acid (precursor of vanillin: 15.000 ton market request)



Coffee powder

Brewed

Beverage

Coffee powder waste

Extraction with hexane/ether

Triglycerides

Solid waste

Biodiesel

Glycerin

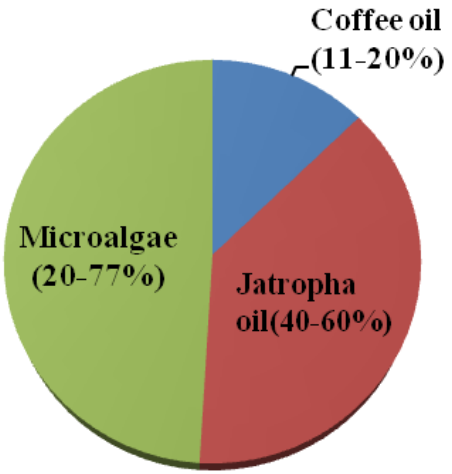
Ethanol

Fuel pellets

H_2

Polymers

Second generation biodiesel!



First generation:
food/land competition

!

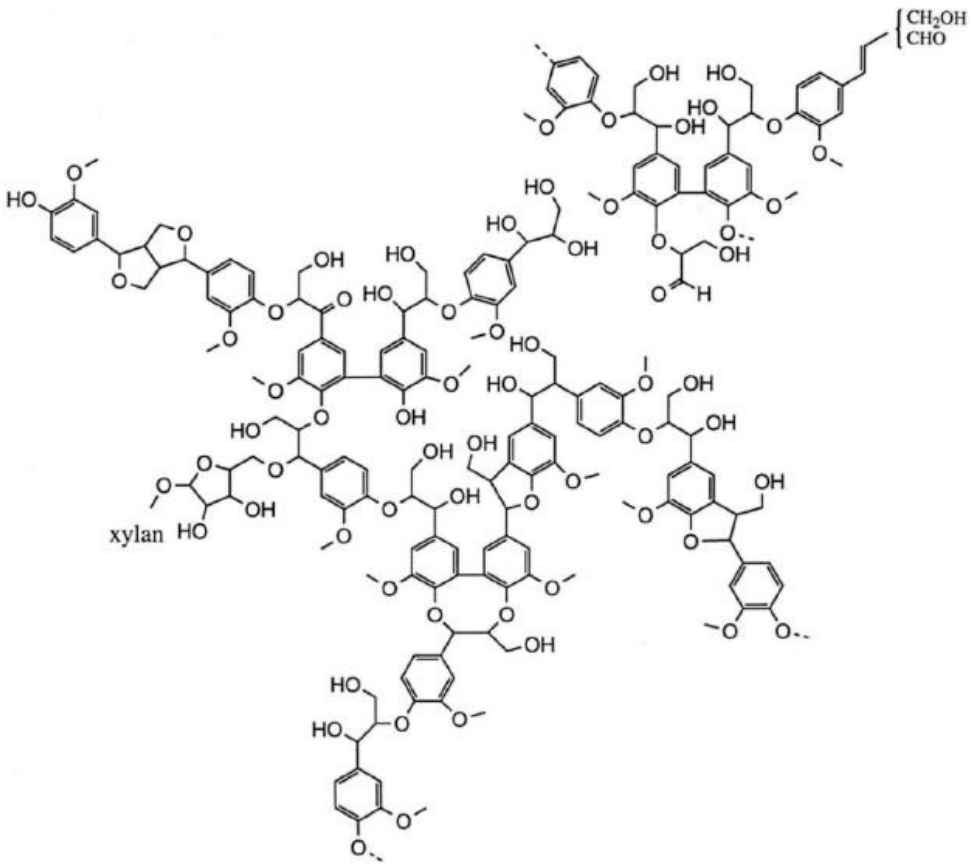
Rapeseed: 37-50%,
Palm: 20%
Soybean 20%

About 20% of the dry weight of the water-extracted coffee grounds is recoverable as oil

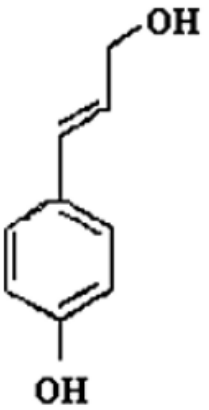
This can add
approximately 1,2 billions L of biodiesel to the world's fuel supply.

Lignin

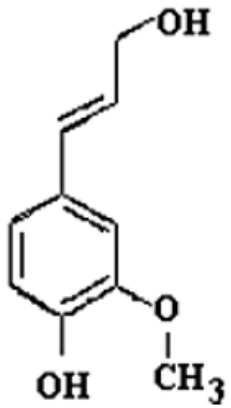
Lignin is a **branched polymer** of **substituted phenylpropane units** joined by carbon-carbon and ether linkages. Biosynthesis of lignin formation proceeds *via* **polymerisation of the free radical forms of precursors**.



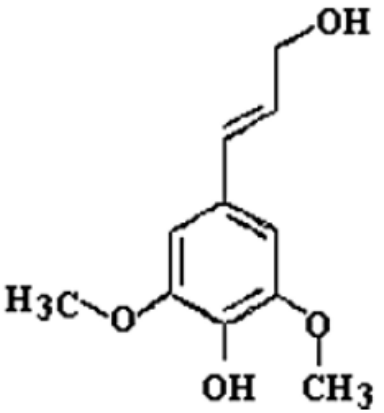
Lignin precursors



p-coumaryl alcohol

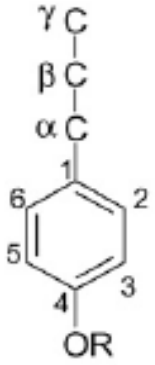


Coniferyl alcohol

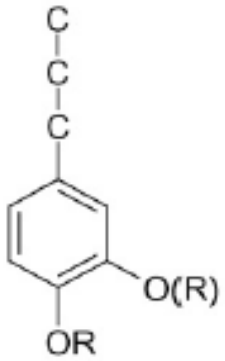


Synapil alcohol

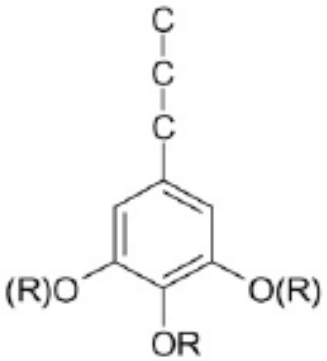
In the final polymer they form:



p-Hydroxyphenyl



Guaiacyl



Syringyl

R=H,alkyl,aryl

Use of lignin

The processing of 140 million tons cellulose and pulp in paper production lead to
50 million tons lignin

About 95% is burned
Only 5% reutilized:

Potential applications of lignin

- Production of vanillin
- Dimethyl sulfide
- Methyl mercaptan
- DMSO
- Dye dispersants
- Pesticide dispersant
- Carbon black dispersants
- Water treatment / industrial cleaning
- Complexing agents for micronutrients (Fe, Cu, Zn, Mn, B) for soils
- Oil-weel cement retarders
- Leather tanning

Enzymes for lignin degradation

Plant laccases and peroxidases catalyse the generation of radical formations.

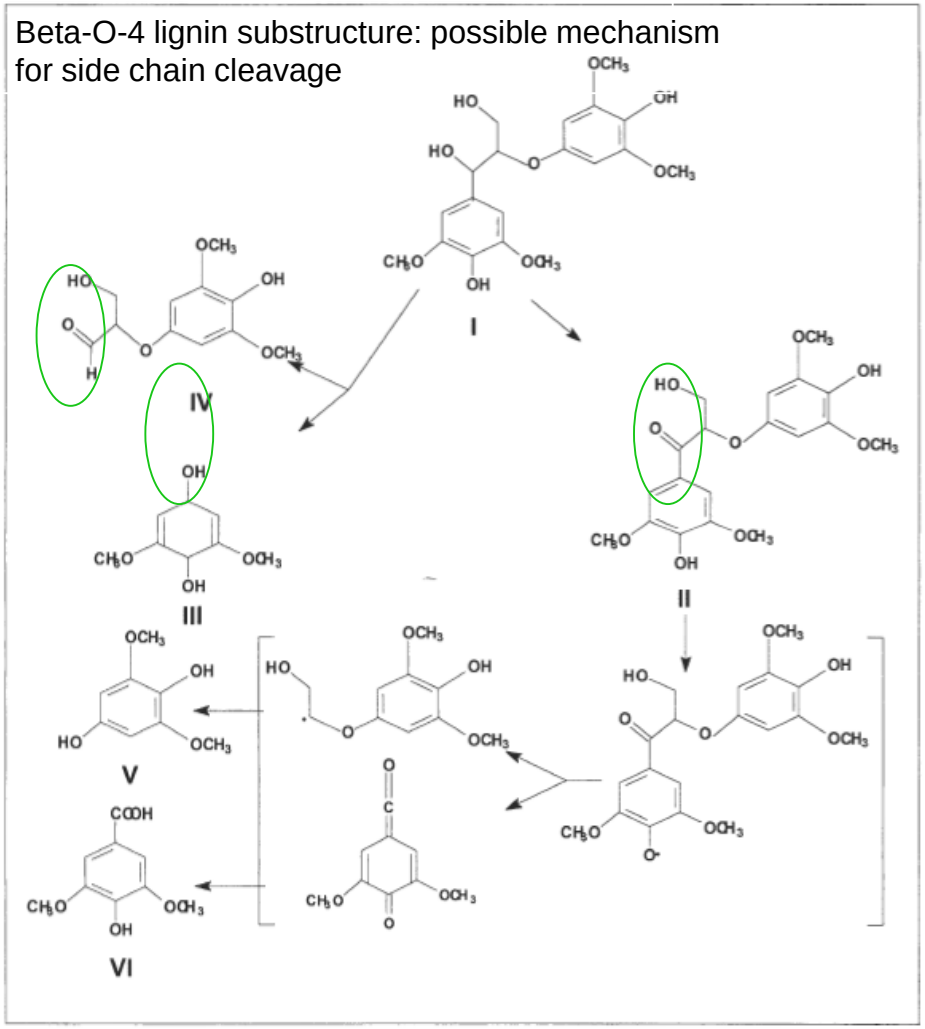
Since **white-rot fungi** are the only degradation, their ligninolytic enzyme

Lignin polymer structure is irregular, enzymes must show lower substrat enzymes in cellulose or hemicellulc

Because lignin consists of interunit enzymes must be oxidative rather

The major consequence of enzymati lignin-related phenols is oxidative c

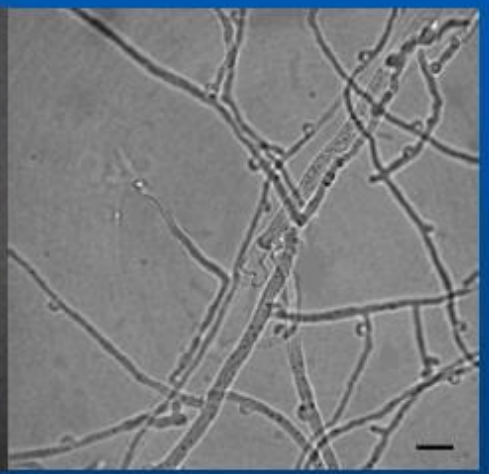
Beta-O-4 lignin substructure: possible mechanism for side chain cleavage



Steccherinum ochraceum 1833

S. ochraceum,
natural fruit-bodies

S. ochraceum 1833,
culture on MEA

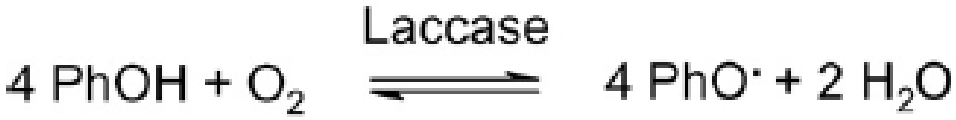


S. ochraceus 1833,
hyphae with clamps

Myasoedova, N.M., Chernykh, A.M., Psurtseva, N.V., Belova, N.V. and Golovleva, L.A. (2008) **New efficient producers of fungal laccases.** *Appl Biochem Microbiol (Russia)* **44**, 84-89.

LACCASES

Benzenediol oxygen oxidoreductases, EC 1.10.3,2: glycoproteins members of the "blue" multi-Cu oxidase family.

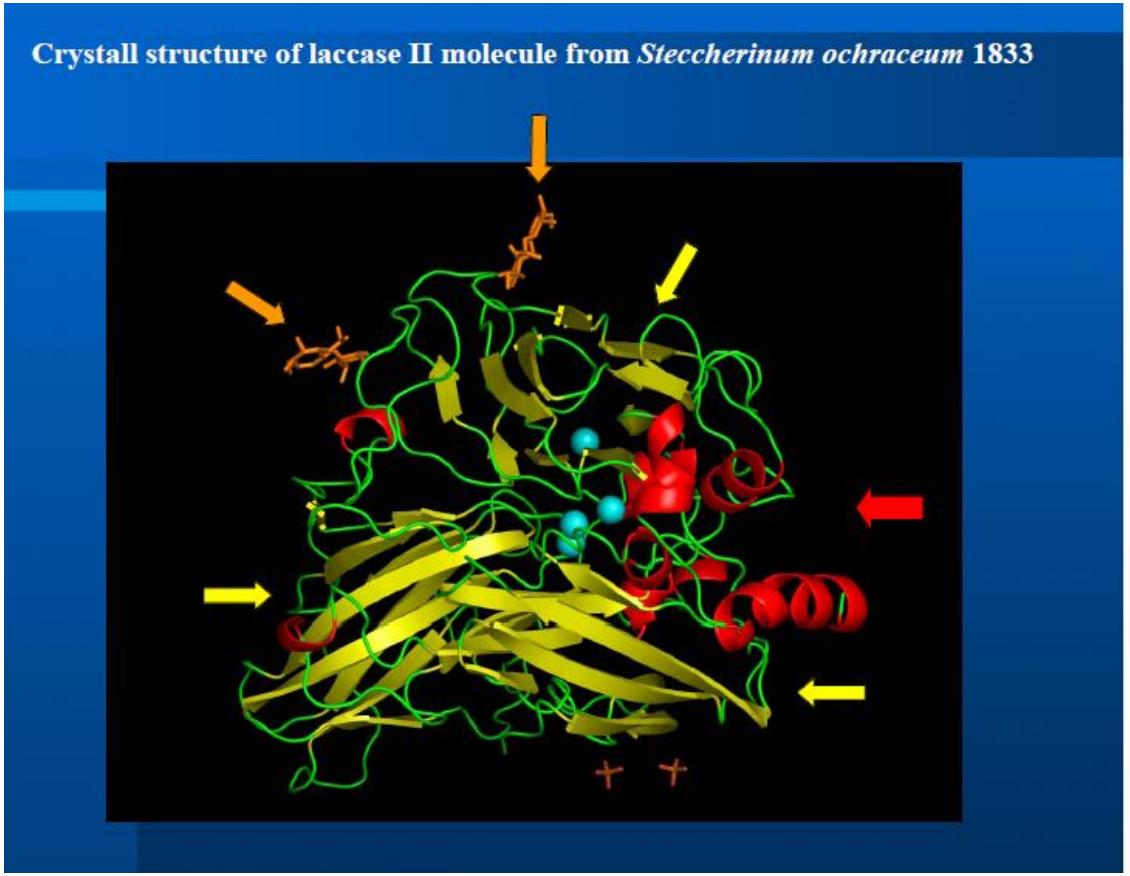


Laccases oxidize aromatic compounds, in particular PHENOLS and AMINES, giving reactive radicals.

"Laccases: blue enzymes for green chemistry" S. Riva *Trends Biotechnol.*, *24*, 219-226 (2006)

Laccases: limitations

1. Laccase is a large and glycosilated molecule (MW 70,000) which cannot penetrate deep into wood

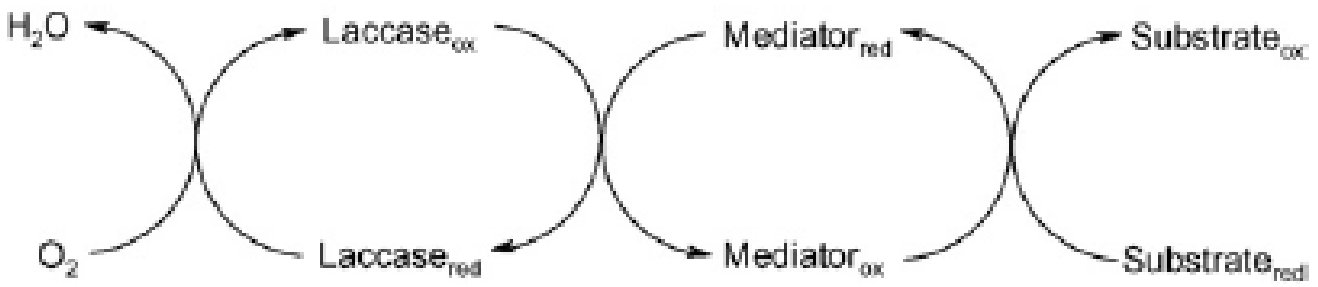


2. Has a rather low-redox potential (~0.5–0.8 V): it is unable to oxidize nonphenolic (C4-etherified) lignin units, which have a high-redox potential (>1.5 V)

3. Because of these limitations, laccase alone can only oxidize phenolic lignin units (<20% of all lignin units in native wood) at the substrate surface.

Ludmila Golovleva, *G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russia*

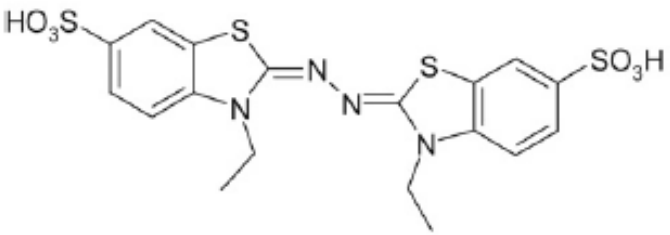
LACCASES and CHEMICAL MEDIATORS



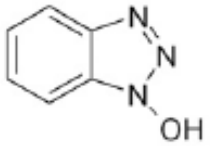
•Therefore, laccase is often applied with an **oxidation mediator**, a small molecule able to extend the effect of laccase to nonphenolic lignin units and to overcome the accessibility problem.

•In these so-called LMS, the mediator is **first oxidized by laccase** and then **diffuses** into the cell wall, oxidizing lignin inaccessible to laccase

Mediators: examples



A



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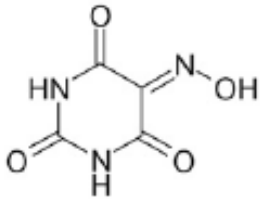

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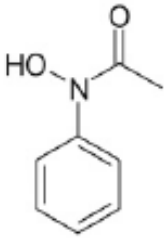

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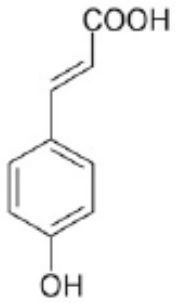

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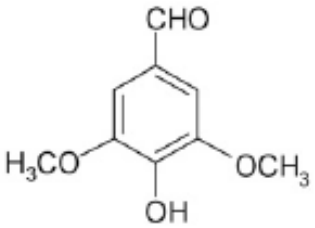

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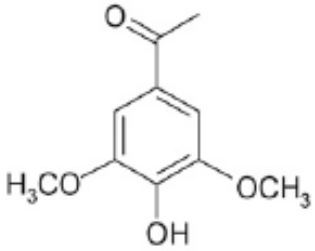


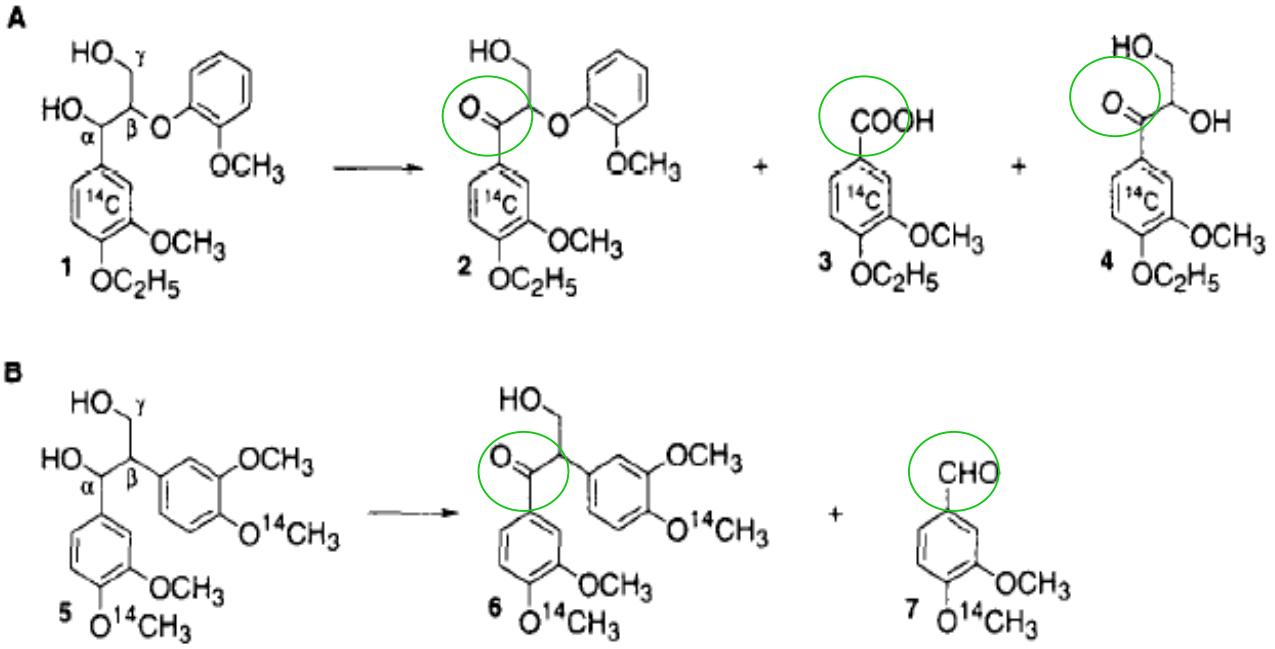
G

p-coumaric acid → Natural mediators

syringaldehyde → Natural mediators

acetosyringone → Natural mediators

Oxidation of non-phenolic lignin models in the presence of mediators



Journal of Biotechnology 81 (2000) 179-188

Biocatalysis for CO_2 transformation:

The most abundant and inexpensive source of renewable carbon on earth is, of course, CO_2, with 36,600 million metric tons of anthropogenic CO_2 emissions per year contributing to greenhouse gas (GHG) and global warming. Nowadays there are mature technologies for **CO_2 sequestration** with large-scale facilities around the world capturing more than 27 million tonnes of CO_2 every year from power generation plants, but also from industrial sectors such as iron and steel, refining, petrochemical, and cement manufacturing.

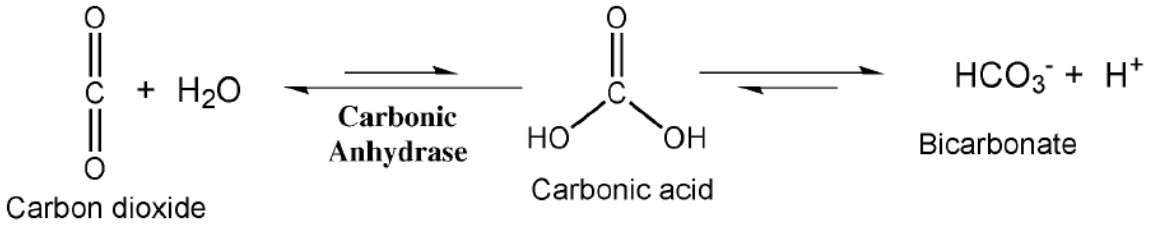
The first CCS project applied to coal-fired power generation commenced operation in 2014

Gorgon Project, Australia
Image courtesy of Chevron

The Sleipner oil and gas project in Norway has been injecting and storing CO_2 for 20 years

Sleipner Platform, North Sea
Image courtesy of Statoil

Enzymes have been investigated as a route for reducing the cost barrier of **carbon capture technologies,** which are the prerequisite for further storage (via gas injection underground for long term storage) or exploitation of CO_2 as a feedstock. One of the many methods currently under development for CO_2 capture is based on the **reactivity of amines, such as mono ethanol amine, in absorber columns at 40-60 °C to form carbamates.** CO_2 is then released by heating the solution at temperatures above 100 °C. This energy intensive process (about 80% of operational costs) requires also large columns to process massive amounts of CO_2. Since the rate determining step for desorption is the hydration of CO_2 to bicarbonate, studies are under development for the use of immobilized carbonic anhydrase to increase the rate of CO_2 desorption. Carbonic anhydrase enzyme is involved in many biochemical processes in nature, such as detoxification pathways, respiration, pH homeostasis and photosynthesis.



Scheme 7 Hydration of carbon dioxide by carbonic anhydrase.

The enzyme allows for the reduction of energy related to the desorption step because it catalyses the fast hydration of CO_2 at lower temperature. The advantage is twofold: lower consume of energy and smaller volume of absorber columns. Because operational temperatures are relatively high, carbonic anhydrase is generally employed in the immobilized form, which displays higher stability upon prolonged storage and enables the recycling of the biocatalyst.

Despite the technological progresses in CO_2 capturing and sequestration, nowadays only a negligible percentage of the anthropogenic CO_2 emissions are transformed for practical use.

CO_2 as feedstock *via* reduction to yield C_1 molecules

The exploitation of CO_2 as feedstock *via* reduction to yield C_1 molecules is in principle an attractive perspective but CO_2 is very a thermodynamically very stable molecule. Actually, the catalytic reduction of CO_2 with H_2 to synthesise methanol is feasible but the reaction has a standard Gibbs free energy change of reaction ($\Delta_r G^o$) of + 0.84 kcal/mol. The reduction has been experimentally carried out using various metal catalysts, which must be activated under harsh conditions both in terms of temperature (150 - 300 °C) and pressures (from 3 to 14 MPa). Moreover, the metal catalysts maintain their activity only in the presence of highly pure feedstocks. These factors justify the impracticability of the thermochemical reduction of carbon dioxide for industrial purposes.

Redox enzymes have the advantage that they are able to catalyze reactions in which conventional chemical catalysts fail. Biocatalysts have been shown capable of catalysing the reduction of CO_2 at ambient conditions and some microorganisms can reduce CO_2 simply by reversing the metabolic pathway reactions. The multiple steps reactions involve usually a formate dehydrogenases that catalyzes the initial reduction of CO_2.

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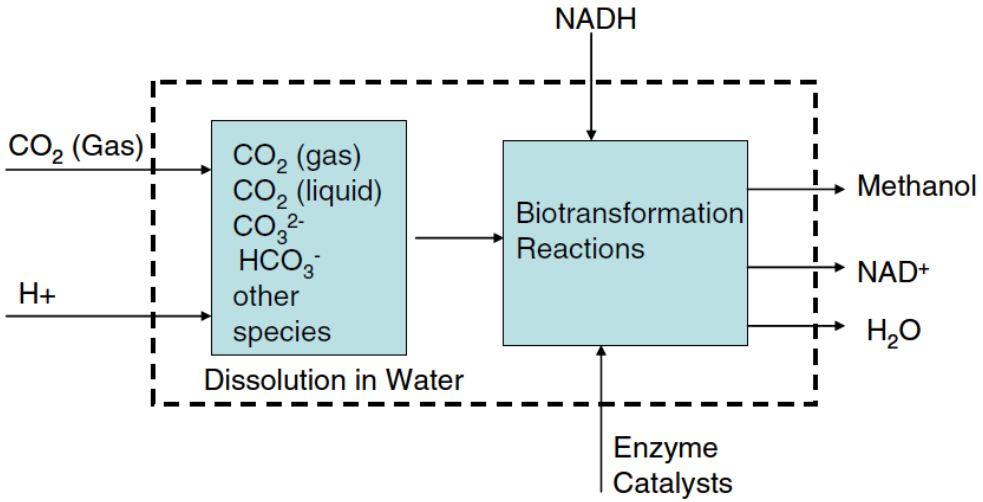
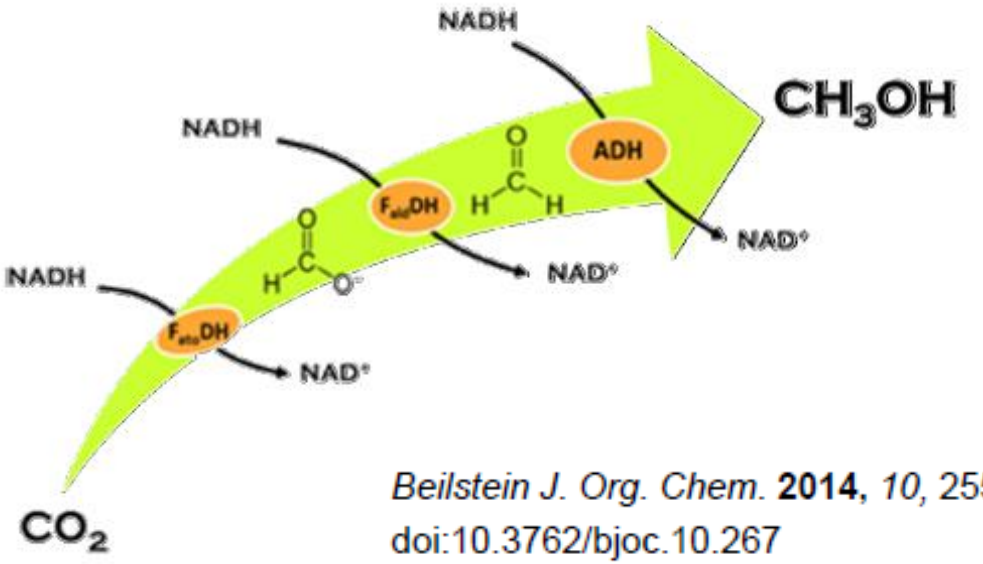
Scheme 1 Carbon dioxide dissolution and transformation processes for enzymatic synthesis of methanol. A black box approach (indicated by the boundaries of *dashed lines*) focusing on the inputs and outputs of the process was applied in the current work



Unfortunately, the use of isolated redox enzymes is hampered by the high cost of the cofactors (e.g. nicotinamide adenine dinucleotide, NAD+) necessary for CO_2 reduction. Major efforts in the field are directed towards the *in situ* regeneration of cofactors.

For instance, **hybrid enzymatic/photocatalytic approaches** were reported for the bioconversion of CO_2 to methanol catalyzed by **three dehydrogenases** (FateDH, FaldDH, and ADH). The enzymes, consume three mol of NADH, which is then **regenerated by exploiting a visible-light-active photocatalytic system made by TiO_2**. The electron (hydride) is then transferred from a H-donor such (e.g. water–glycerol solutions) to NAD+ with the assistance of a Rh(III)-complex. Globally, the process allows for the production of 100-1000 mol of methanol starting from one mol of NADH .



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Since the reduction of carbon dioxide requires the energy to proceed uphill to the product of reaction, ideally such source of energy should be renewable and provided either through direct routes, such as photons and electrons, or indirectly by using high-energy chemical molecules as hydrogen.

One emerging field of research is the study of **bioelectrochemical systems (BESs)**, which are either able to oxidize organic compounds or to produce hydrogen by reducing protons.

The transfer of electrons occurs through interactions that are established between electrodes and these specific biocatalysts. The electrogenic or electrically-active bacteria are able to perform ‘extracellular electron transfer’ (EET) and they have been isolated from different environments, including extreme environments.

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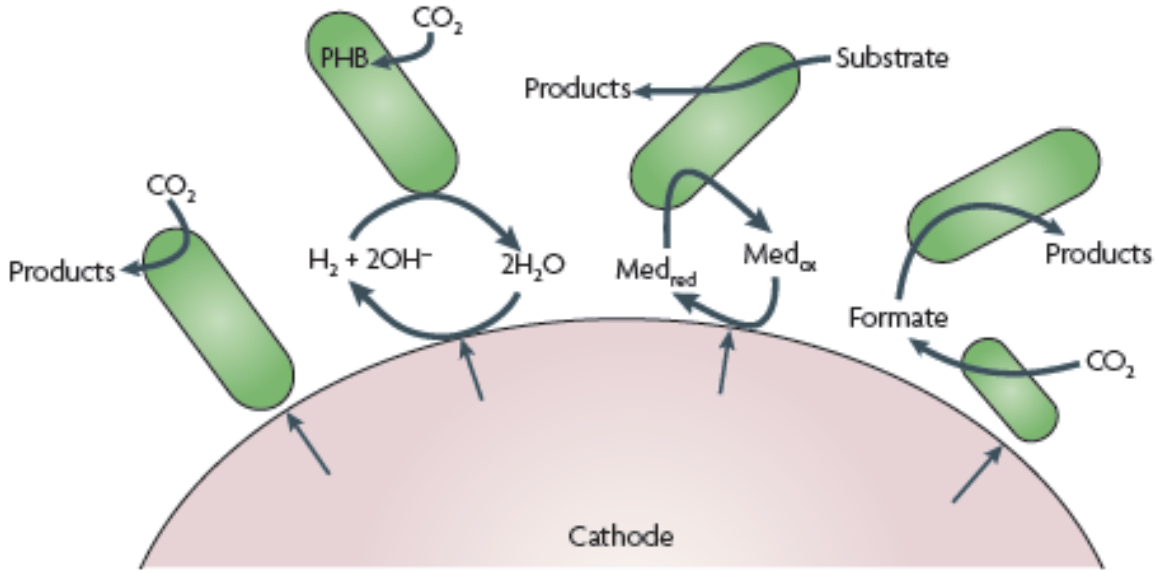
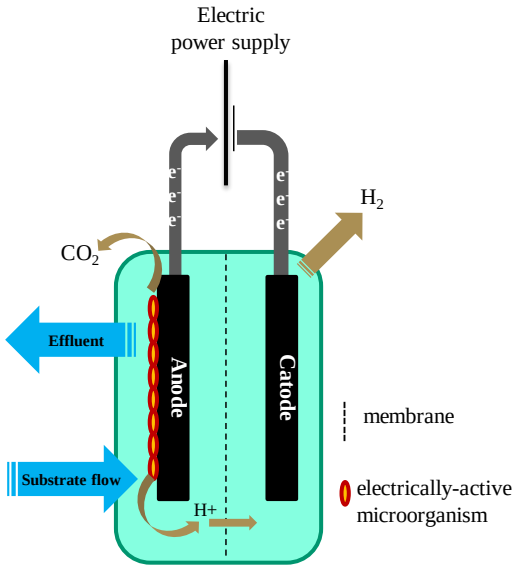


The use of whole microorganisms in bio-electrosynthetic systems is generally preferred because enzymes adsorbed on electrodes lack of long-term stability, although they provide higher reaction specificity and controllability

BESs work as any other electrochemical cell (e.g. a battery), where an anode and a cathode are connected through an external wire that closes the electrical circuit. Optionally, a membrane separates the two electrodes.

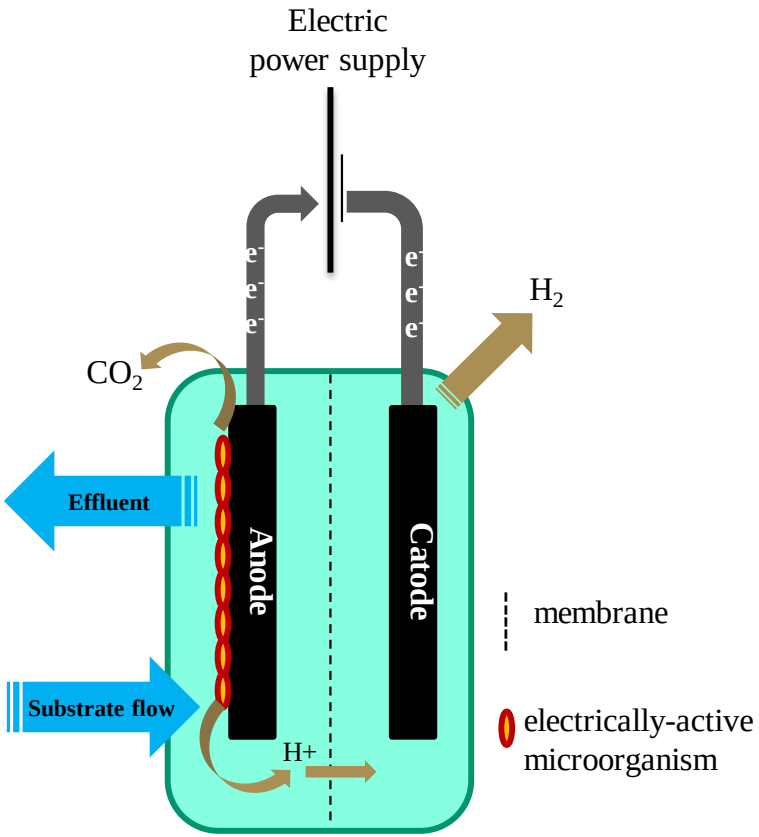
Electrogenic microorganisms oxidize organic substrates at the anode and then transfer electrons from inside their cell to the electrode.



At the same time, the microorganisms release protons in the solution, where the two electrodes are submerged, and also CO_2, which can be captured. The electrons flow to the cathode, where a reduction reaction occurs.

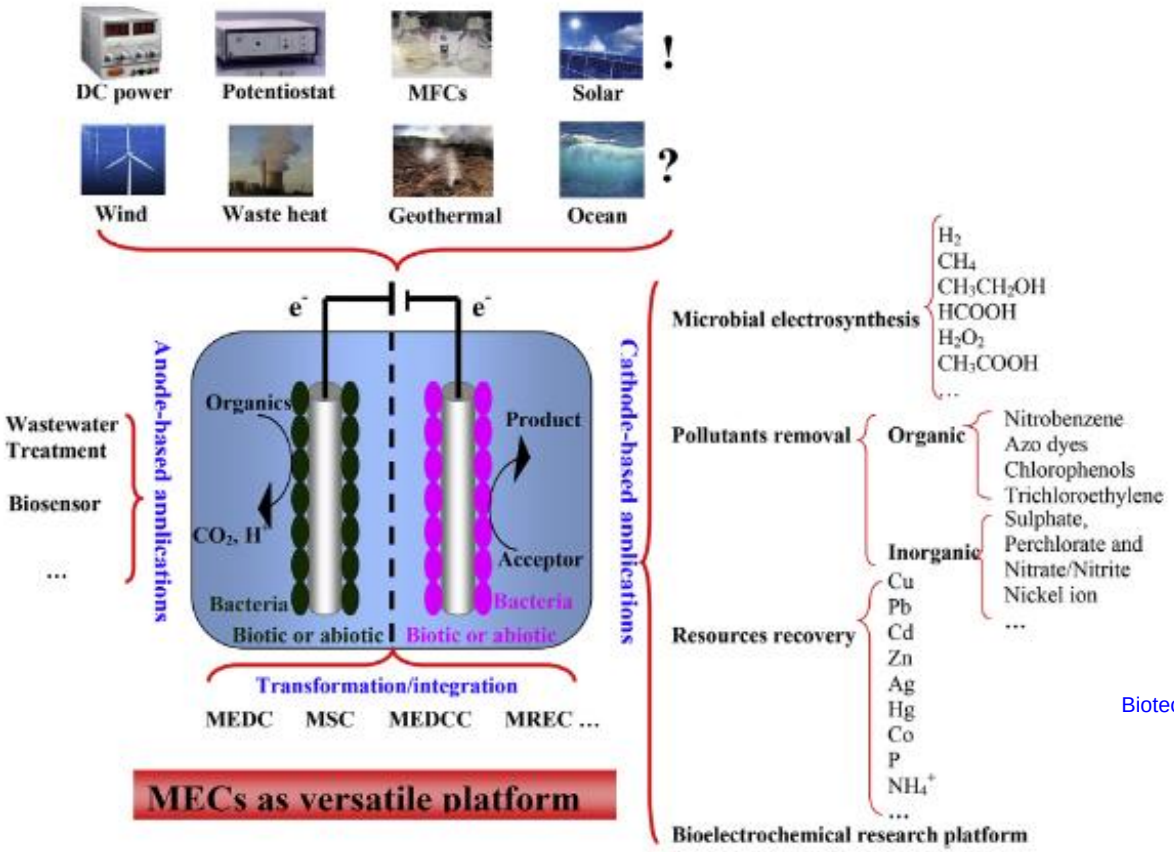
A first type of BESs is represented by the Microbial fuel cells (MFCs), which operate under aerobic conditions. When electrons reach the cathode they combine with oxygen and protons to produce water. MFCs produce electric power, which derives from the external circuit that carries the electronic flow.

Alternatively, when the cathode operates under anaerobic conditions, the electrons reduce protons to form hydrogen. **This BES configuration is referred as MEC or Microbial Electrolysis Cell** and it requires, besides the energy produced by the same microorganisms, some extra energy supply to accelerate the kinetics of substrate conversion or to drive reactions that are thermodynamically unfavourable.



MECs could be a promising means for **producing renewable hydrogen**, an attractive and sustainable energy carrier. MECs have the advantage that they require a limited amount of energy to treat wastewater and the final energy balance is positive since the energy contained in the hydrogen produced through the process counterbalances the electric power supplied for microbial electrosynthesis operation. They have also a four-fold higher hydrogen productivity as compared to conventional processes based on microbial fermentations and are efficient in the treatment of diluted concentration of organic components at very mild temperature (< 20 °C).

-electricity-mediated microbial hydrogen production from waste streams
-reduces the electric energy cost for hydrogen production in contrast to direct water electrolysis.
Other applications: chemicals synthesis, recalcitrant pollutants removal, resources recovery,



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