UNIVERSITÀ DEGLI STUDI DI PADOVA

BIOFUELS FROM PHOTOSYNTHETIC ORGANISMS

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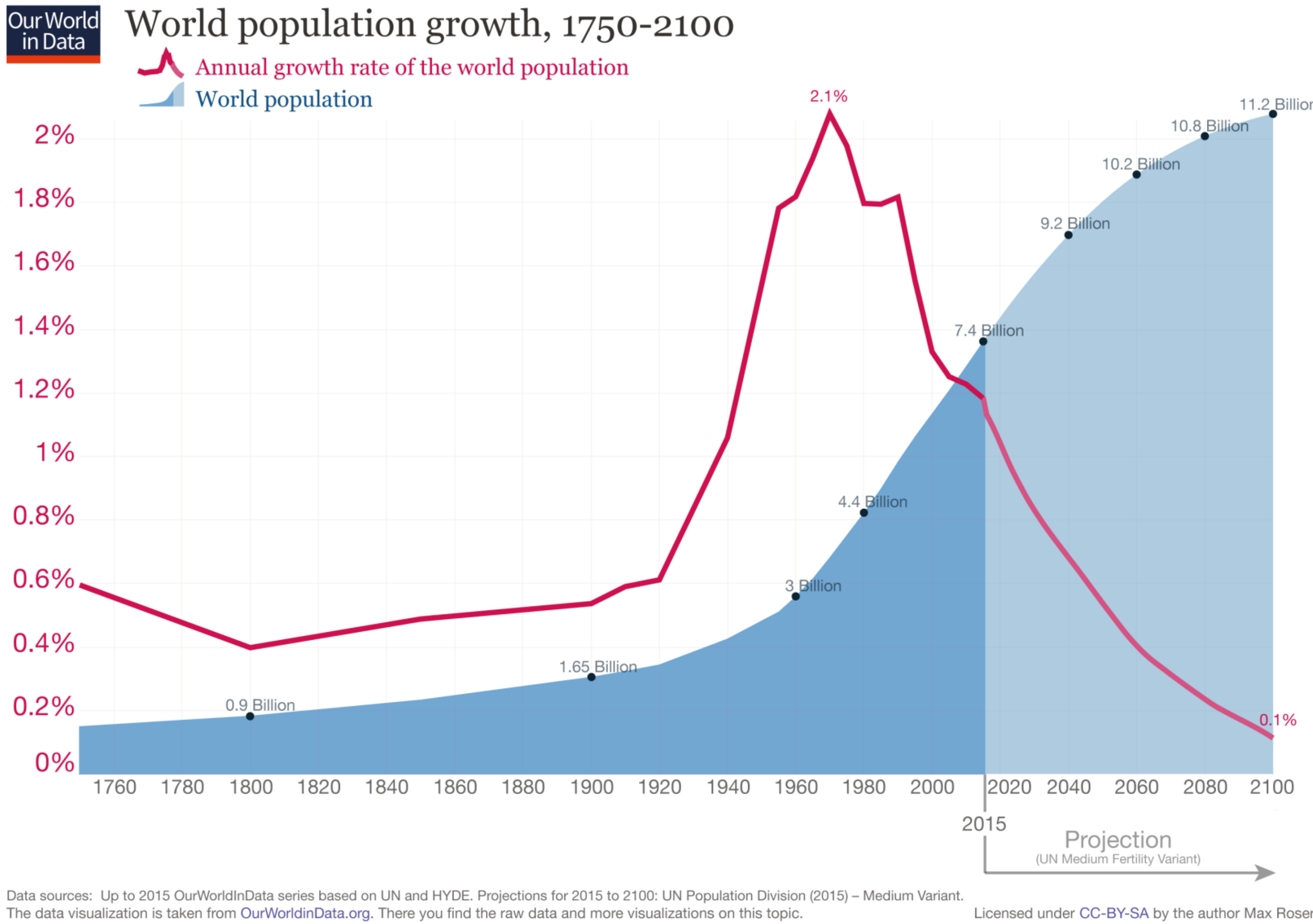
Outline

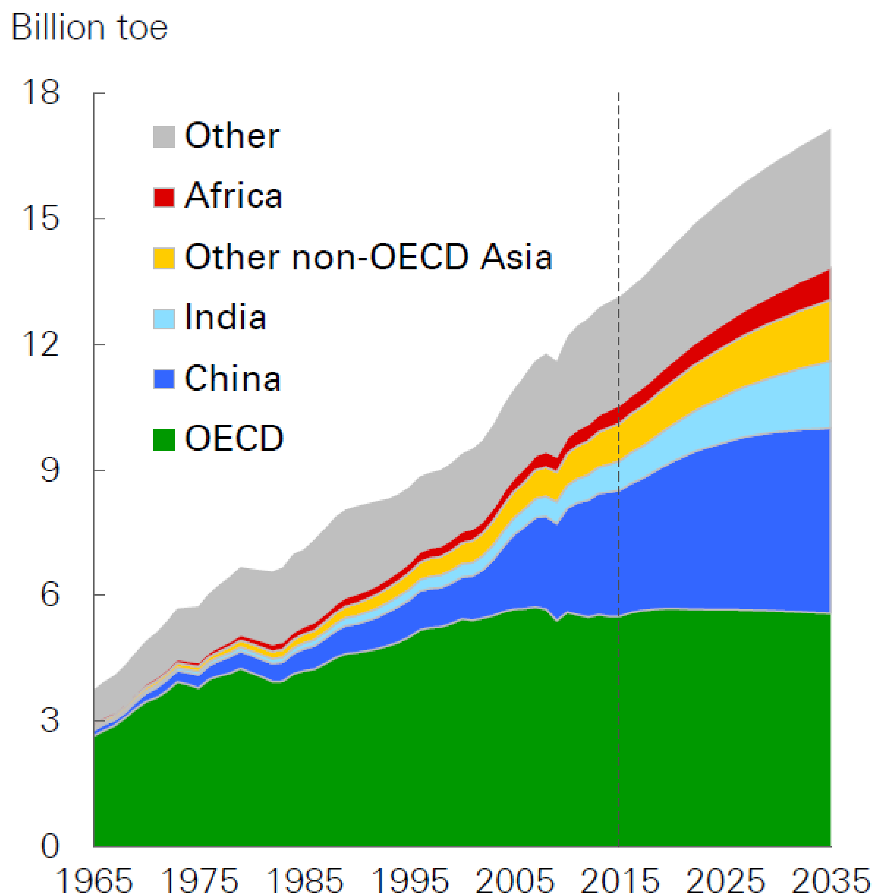
1. Introduction to biofuels
2. Production of BioetOH – Biotechnological challenges
3. Production of Biodiesel – Biotechnological improvements
4. Using algae for the production of biofuels.
5. Perspectives

We should use them more in the future..



.. Increase in population will lead to increase of food and energy demand

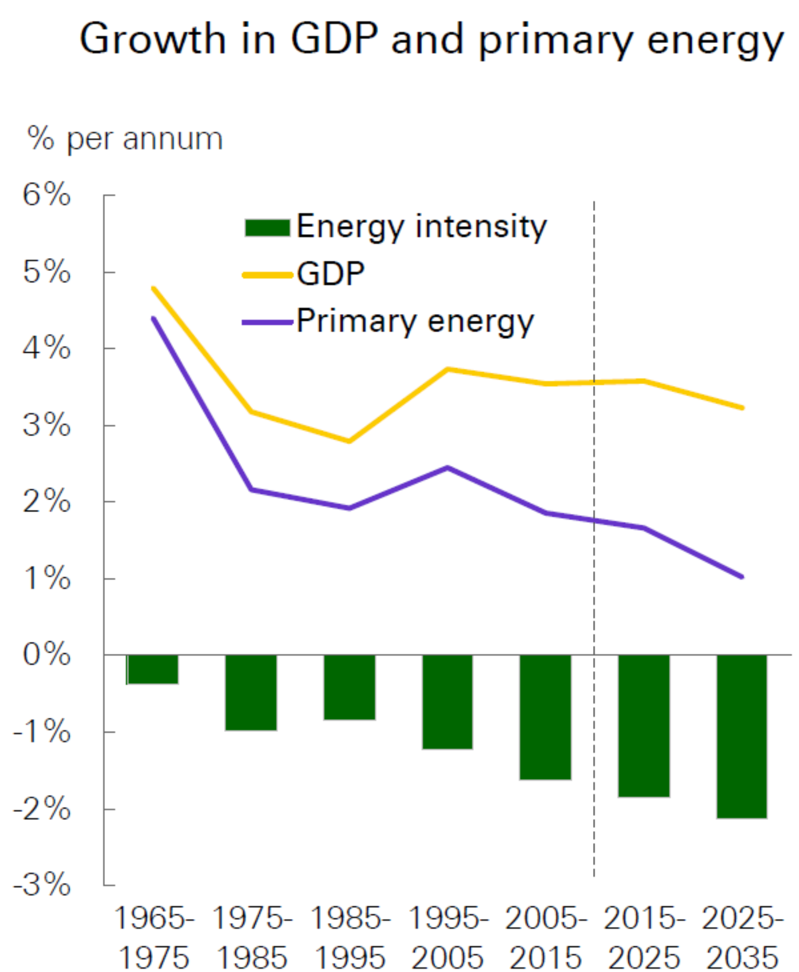
Energy consumption by region



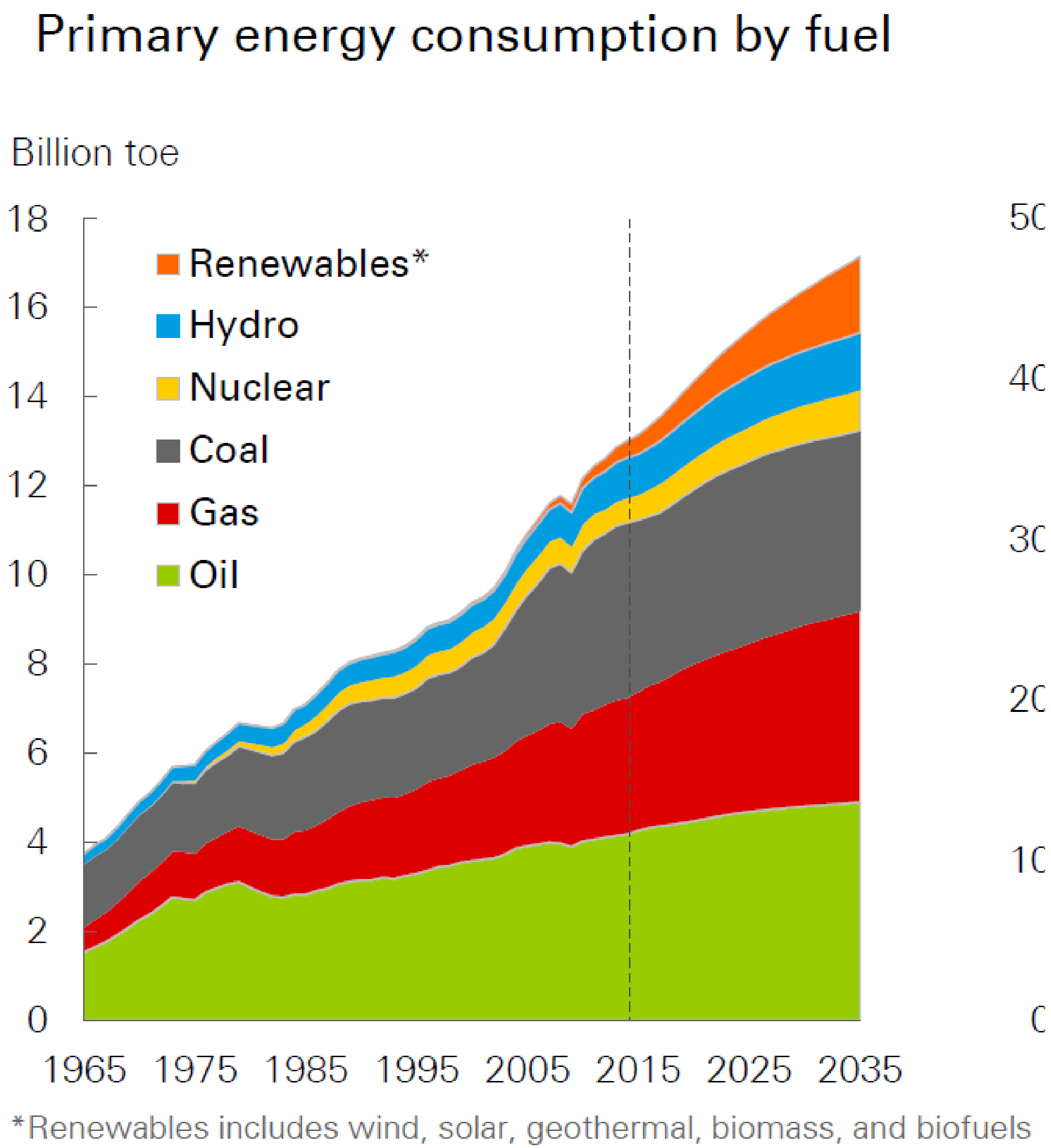
Organization for Economic Co-operation and Development (OECD), OCSE – in Italian

Toe = tons of oil equivalent

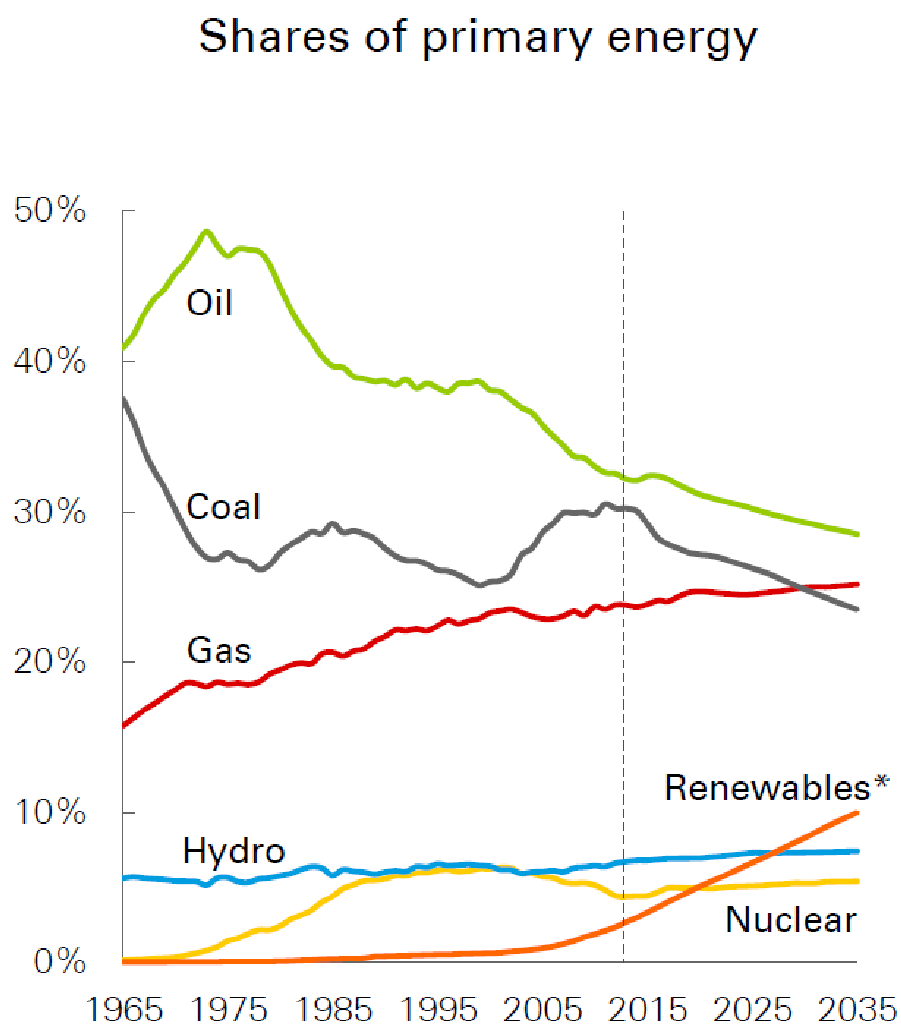
In general we are becoming more efficient in using energy. But the overall demand is still strongly growing



We still heavily relies on fossil fuels



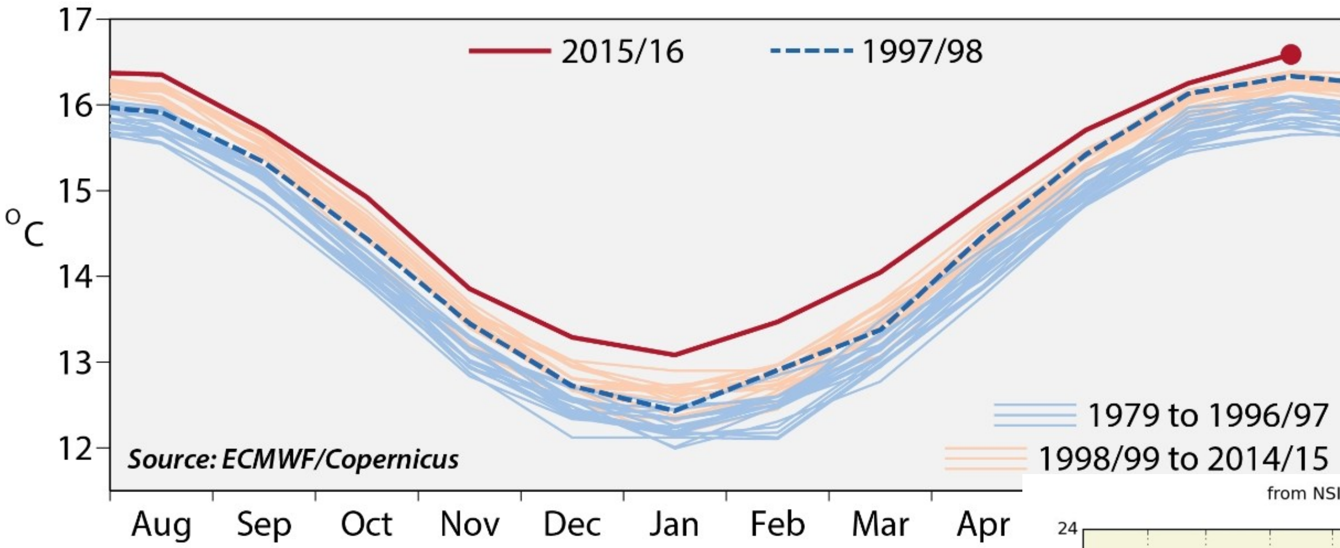
Renewables energy sources are increasing fast but still cover a limited share



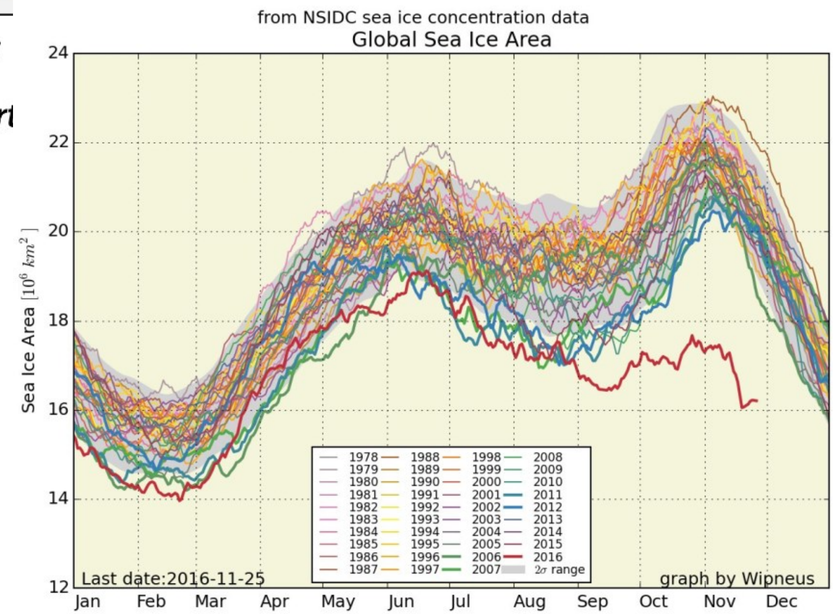
- Even so, oil, gas and coal remain the dominant sources of energy powering the world economy, accounting for more than three-quarters of total energy supplies in 2035 (down from 85% in 2015).

The challenge of reducing CO_2 emissions

Reduce CO_2 emissions is a even more pressing challenge



Monthly global average air temperature at the Eart
from January 1979 to July 2016

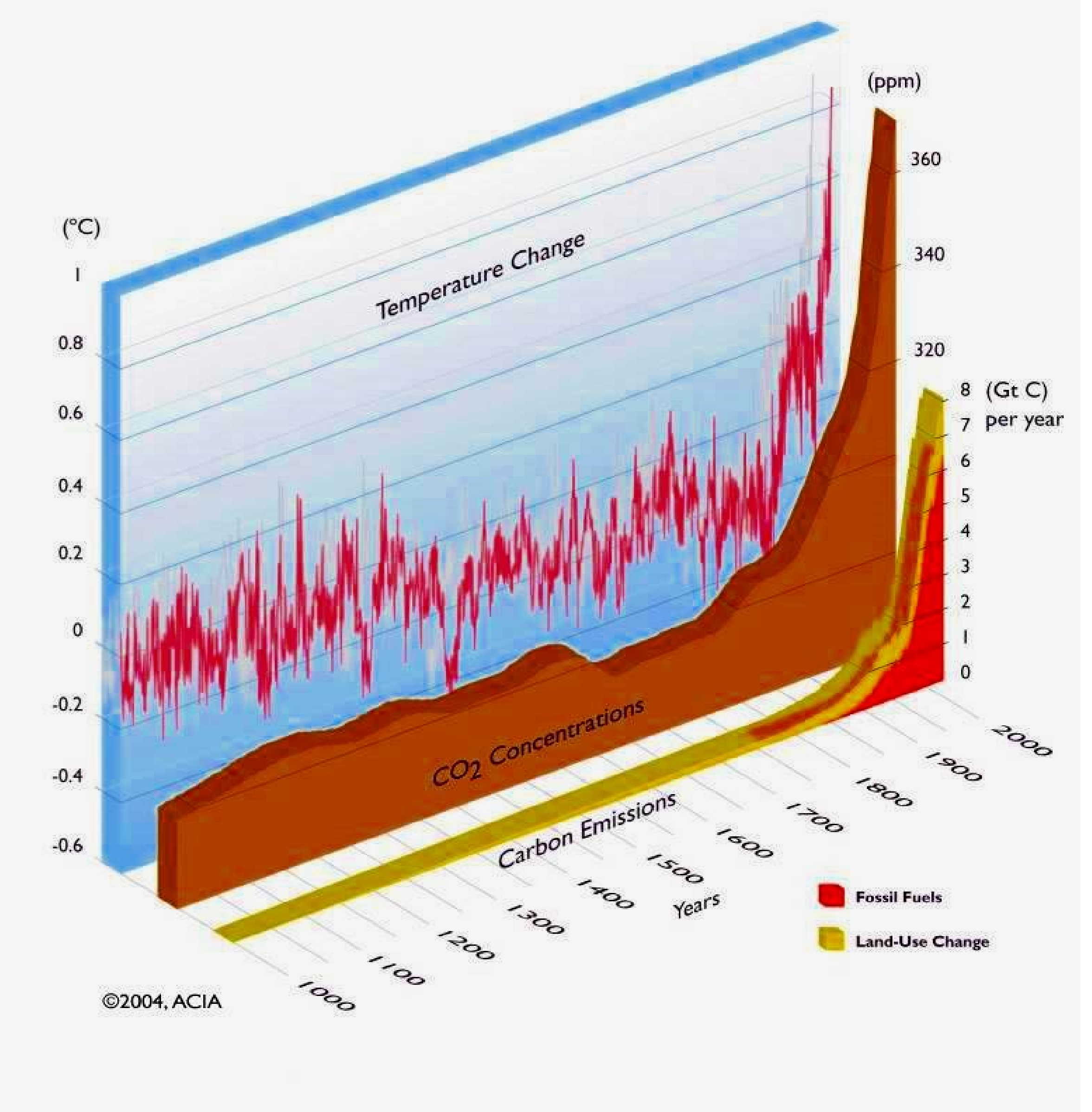


The Need of Renewable source of energy

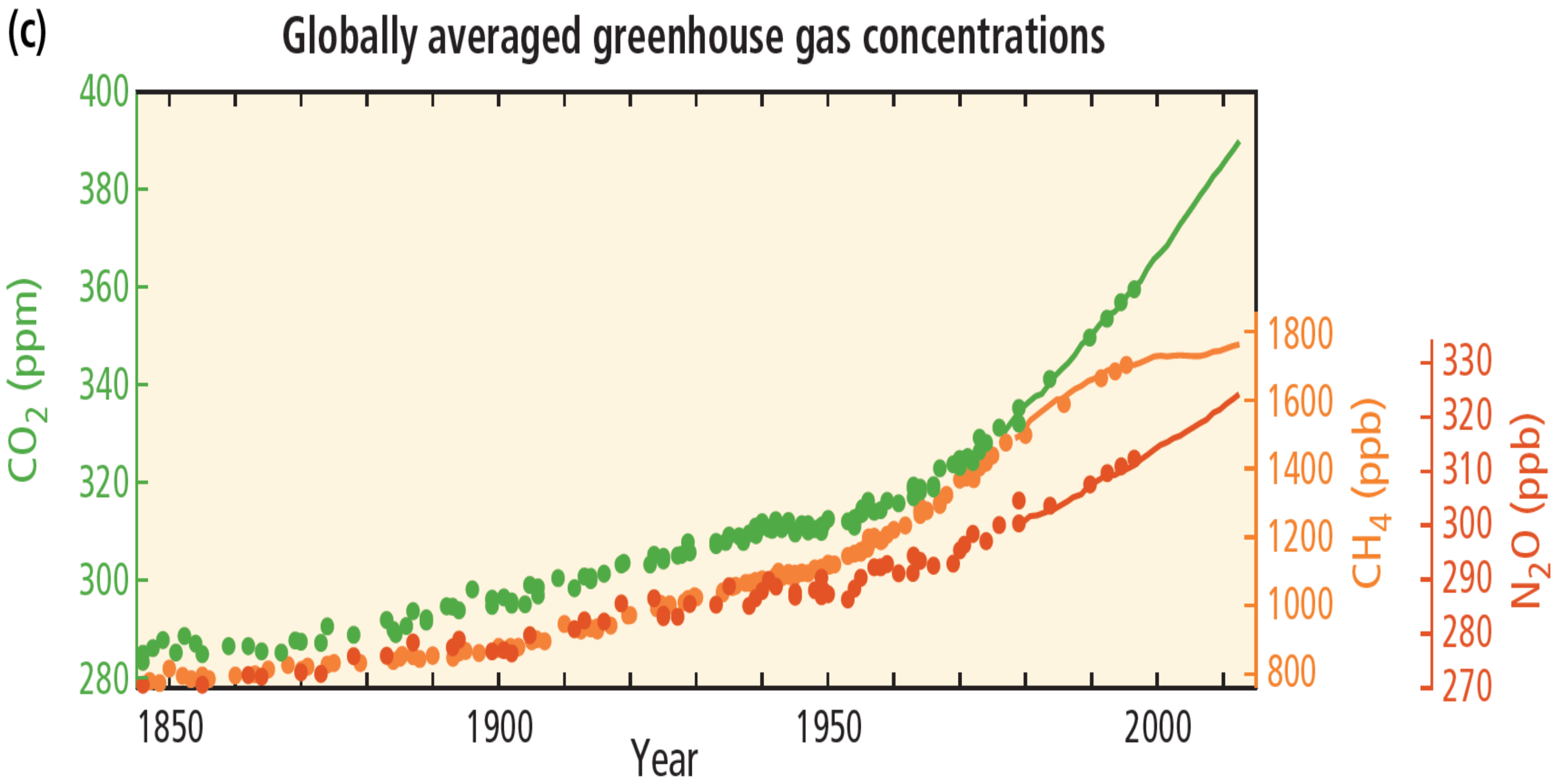
3. “Environmental” argument

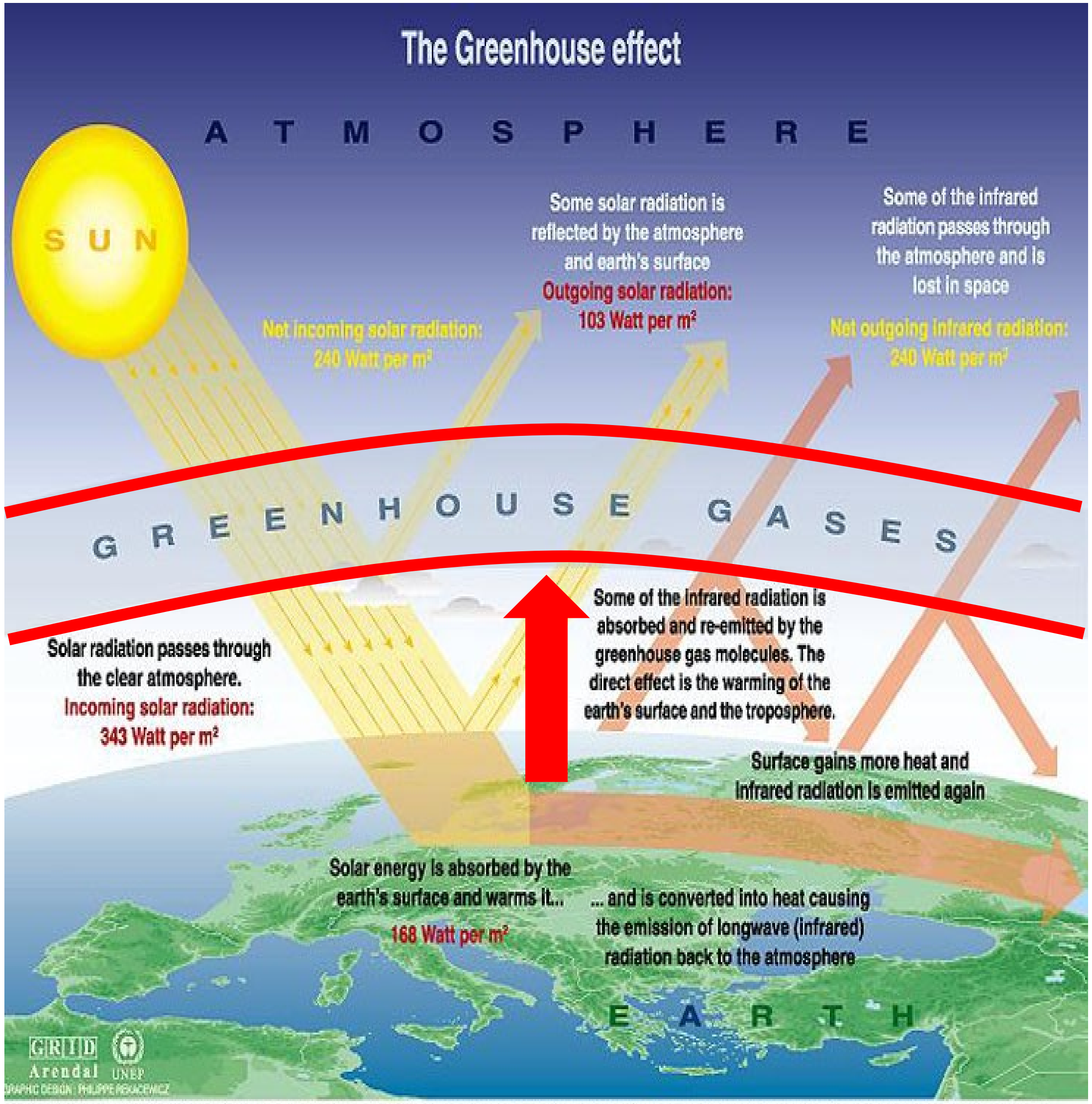
Massive use of fossil fuels causes a large increase in CO_2 concentration in the atmosphere

This Greenhouse gas arguably leads to an increase in global temperature



GREENHOUSE GASES CONCENTRATION IN ICE CORES FLUCTUATED BELOW 300 ppm IN THE LAST 400000 YEARS BEFORE 1900 WHEN THINGS CHANGED





Sources: Okanagan university college in Canada, Department of geography, University of Oxford, school of geography; United States Environmental Protection Agency (EPA), Washington; Climate change 1995, The science of climate change, contribution of working group 1 to the second assessment report of the intergovernmental panel on climate change, UNEP and WMO, Cambridge university press, 1996.

Similar to a greenhouse, at 10 km height in the atmosphere there is something behaving like a glass surface in a greenhouse.

These gases are necessary for life on earth.

Mankind produced and encreased these gases in the atmosphere

CLIMATE CHANGE IS LIKELY IRREVERSIBLE

A&E › Clima

Fai la Ricerca | Vai a ANSA.it

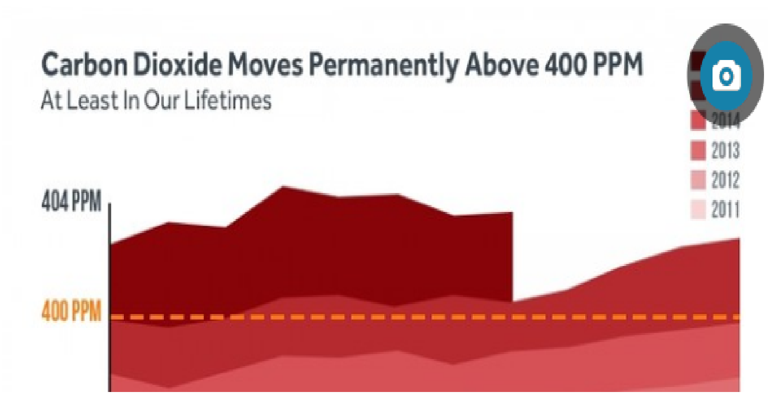
Animali | Natura | Clima | Vivere Green | Mobilità | Rifiuti&Riciclo | Energia | Acqua | Inquinamento | Green Economy | FOTO | VIDEO

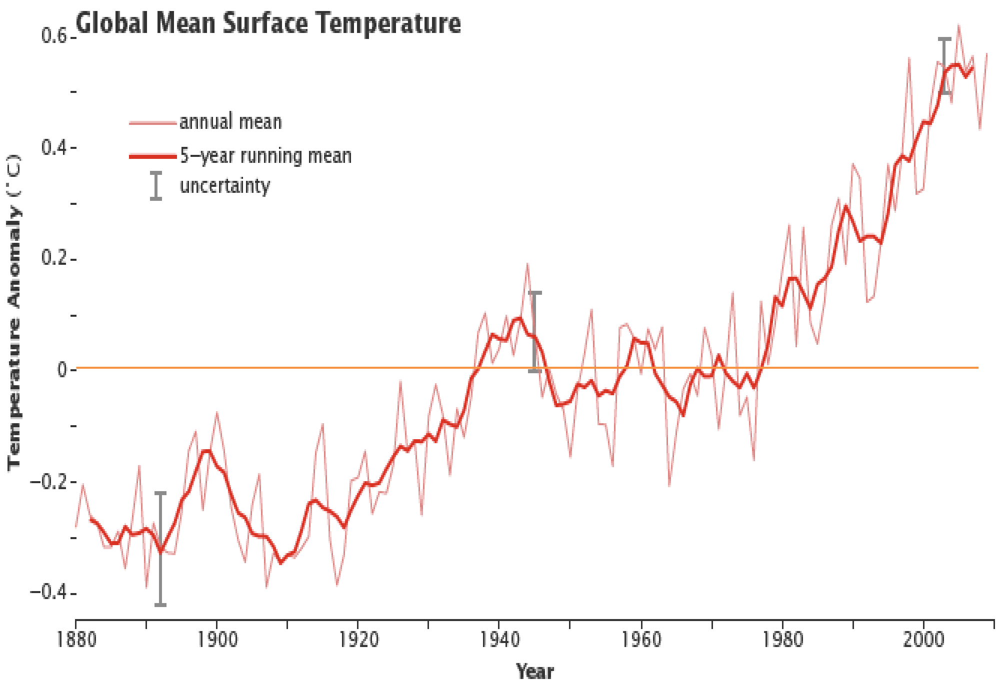
ANSA.it › Ambiente&Energia › Clima › Clima: a settembre CO2 oltre 400 ppm, permanentemente

Clima: a settembre CO2 oltre 400 ppm, permanentemente

Ricercatori, nel mondo mai più sotto soglia 'simbolo'

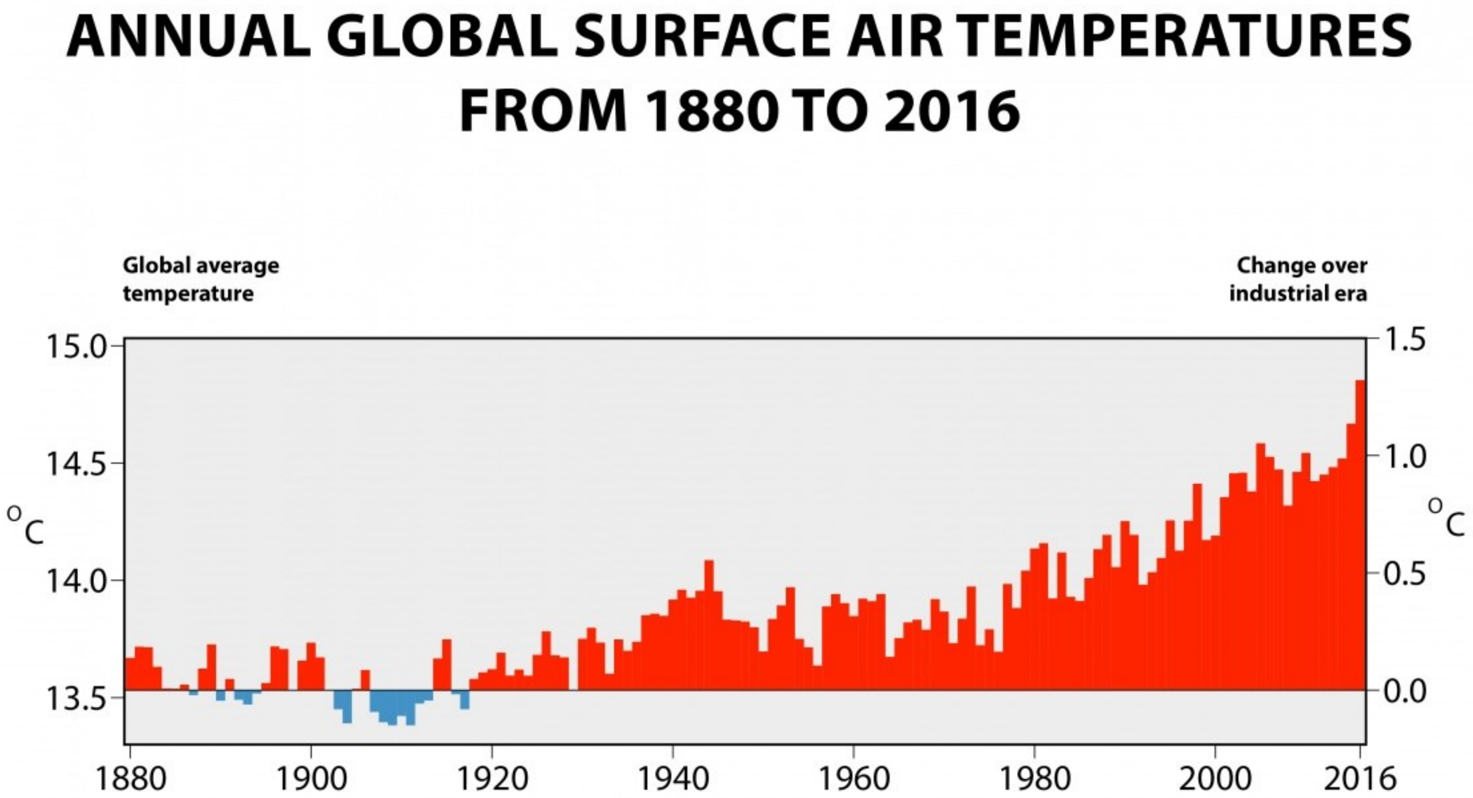
Redazione ANSA ROMA 28 settembre 2016 16:42 Scrivi alla redazione Stampa





Observed globally averaged combined land and ocean surface temperature anomalies (relative
to the mean of 1986 to 2005 period, as annual and decadal averages) with an estimate of decadal mean uncertainty included for one data set (grey shading).

We already experience increased temperatures



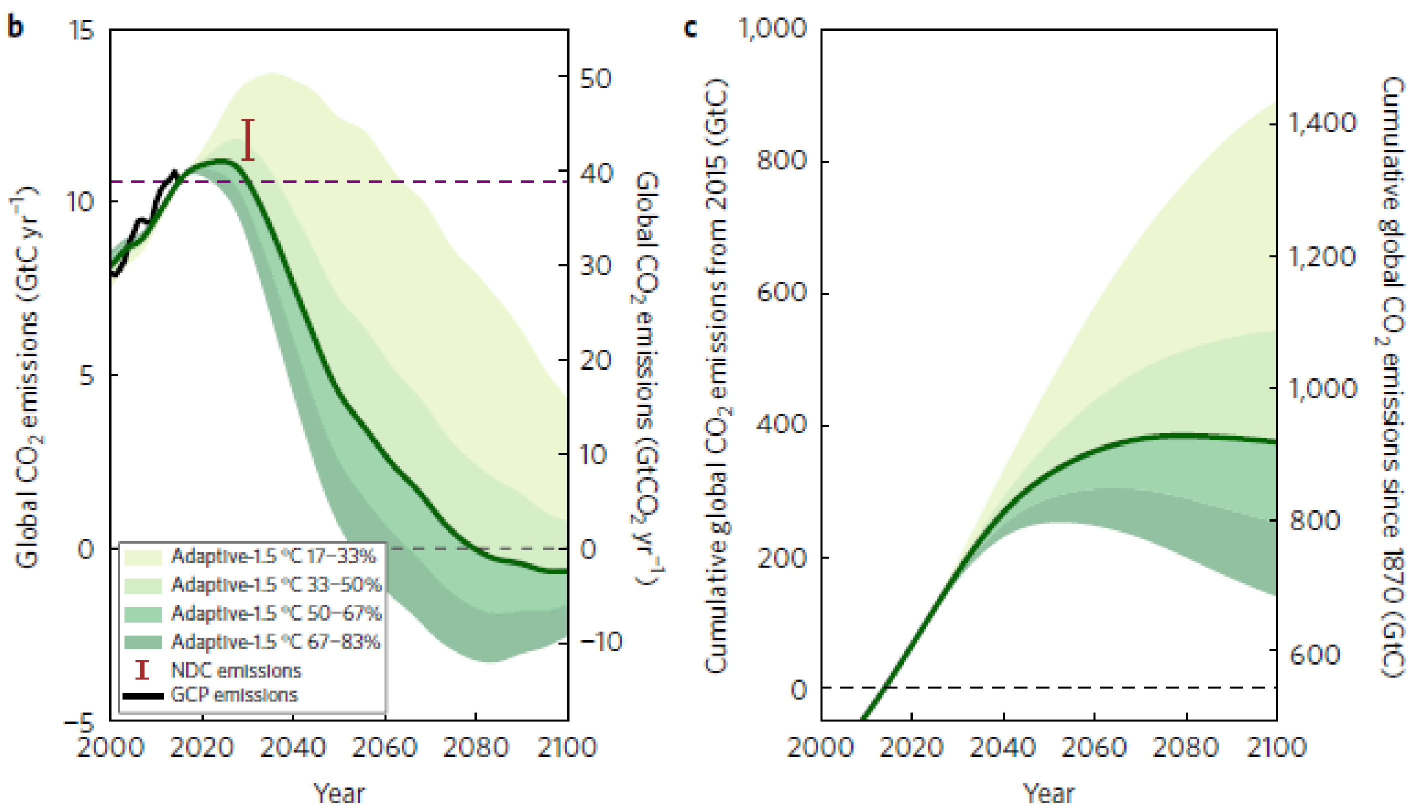
Present increase: + 0.8 ° C

There is the strong risk that it is already too late to limit the increase to 2°C https://www.nature.com/ngeo/journal/vaop/ncurrent/pdf/ngeo3031.pdf

nature geoscience
ARTICLES
PUBLISHED ONLINE: 18 SEPTEMBER 2017 | DOI: 10.1038/NGEO3031

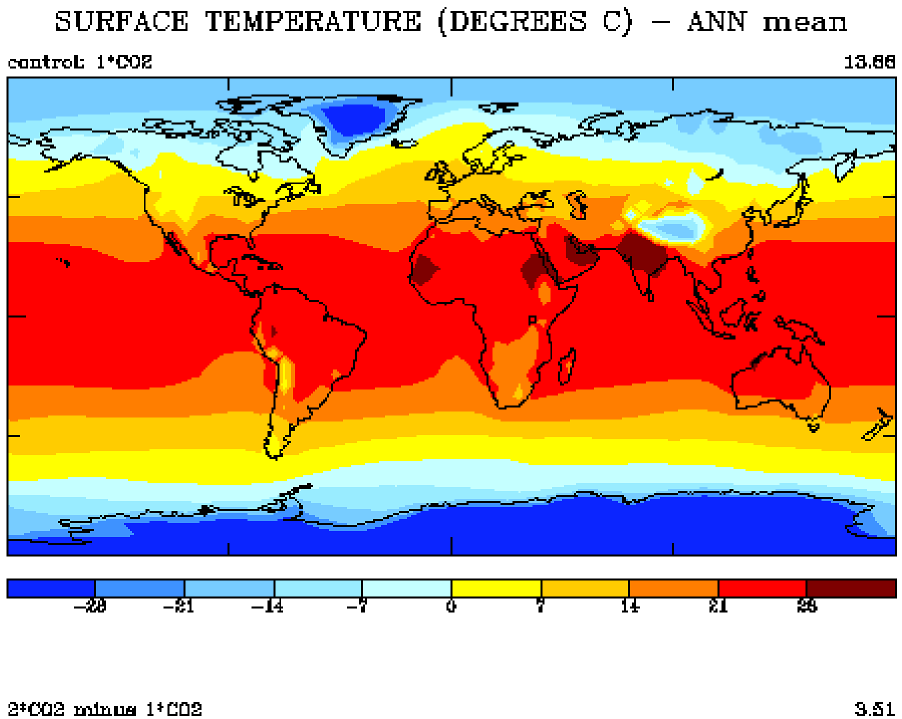
Emission budgets and pathways consistent with limiting warming to 1.5 °C

Richard J. Millar[1,2]*, Jan S. Fuglestvedt[3], Pierre Friedlingstein[1], Joeri Rogelj[4,5], Michael J. Grubb[6], H. Damon Matthews[7], Ragnhild B. Skeie[3], Piers M. Forster[8], David J. Frame[9] and Myles R. Allen[2,10]



Why a few degrees more can be a big deal:

Global average temperature is around 15 ° C, so 3-4° C is a 25% increase



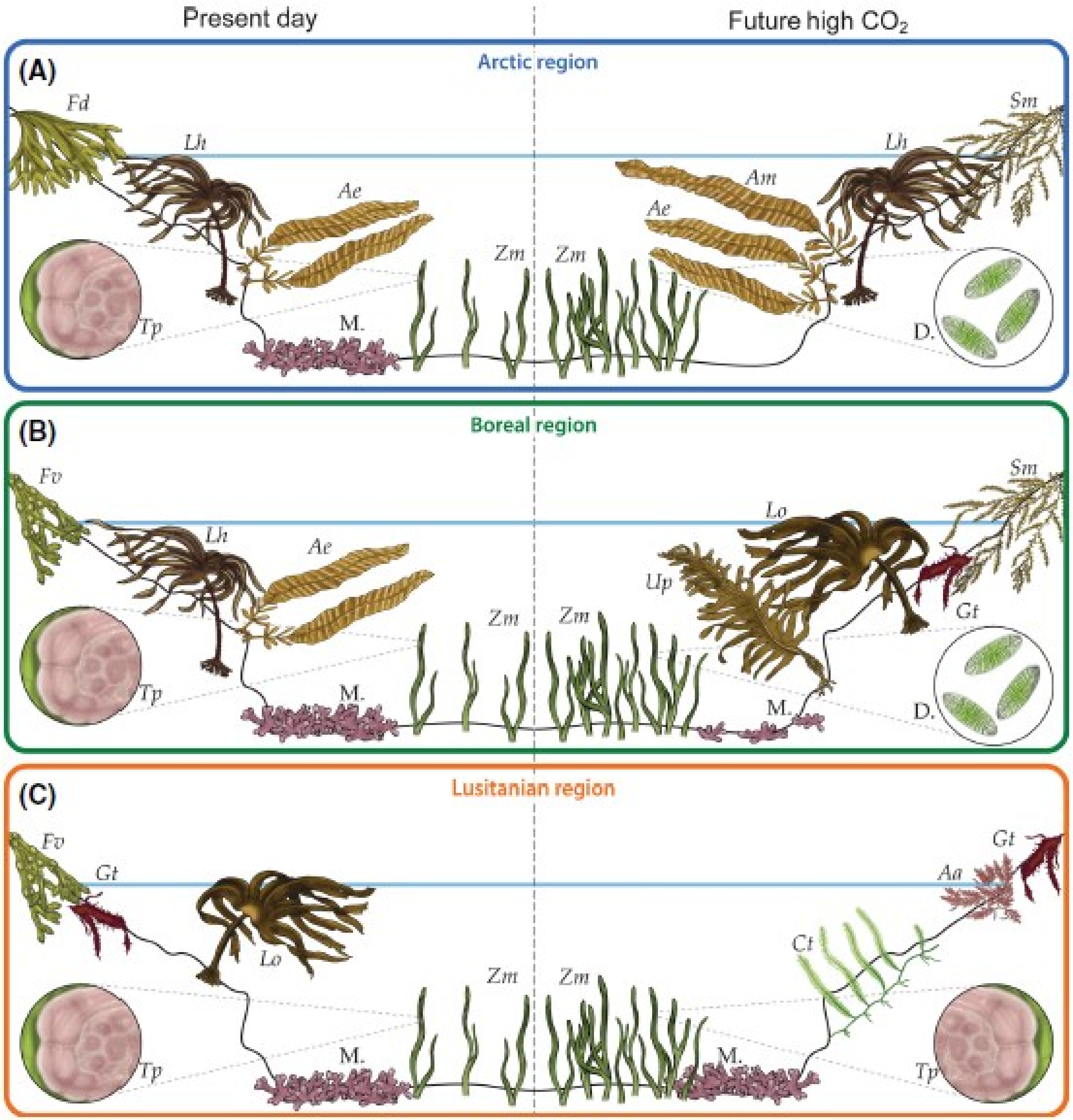
Effects are particularly strong on oceans

Warming is only one of many facets or parts of climate change

OTHER GRADUAL CHANGES:

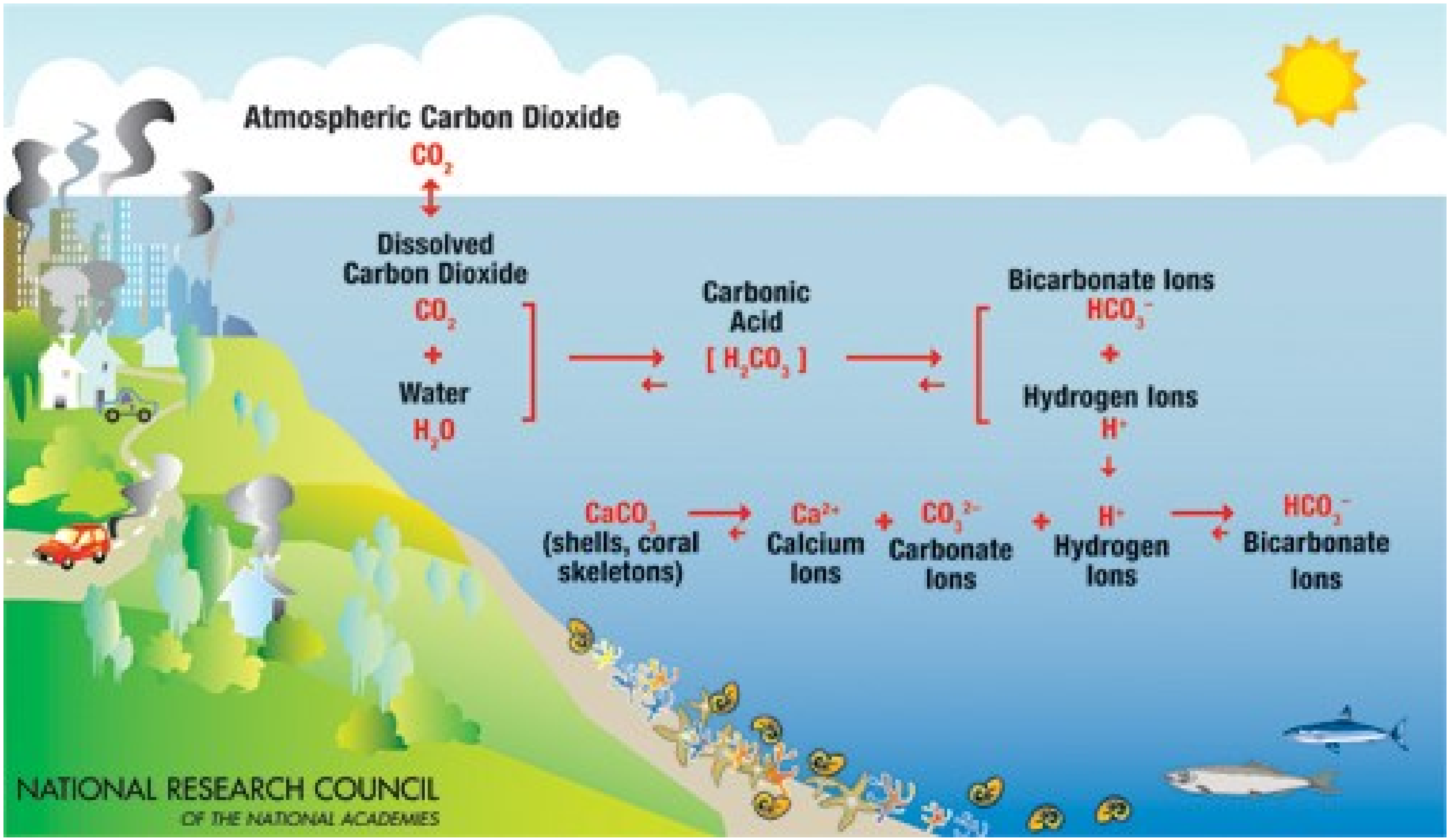
- Rain fall systems change (same area get more, some less than before)
- Ocean rise
- Oceans get warmer
- Oceans get more sour because CO_2 dissolves more into them (not good for fish, O2 content, coral reefs)
- Melting of glaciers (e.g. Himalaya feeding 1 billion people from China to Bangladesh)
- Higher probability of abrupt weather phenomena

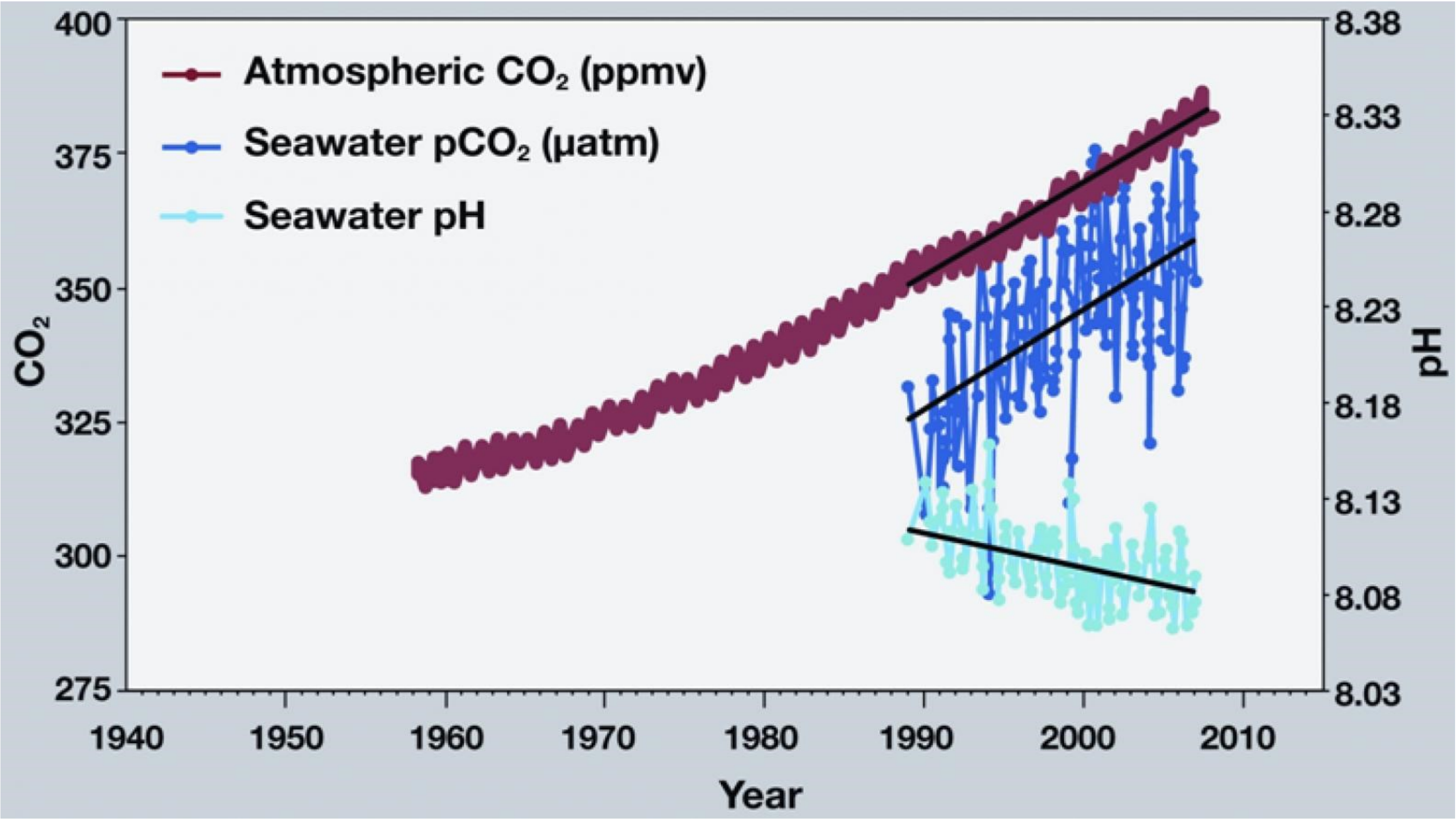
Ecological consequences



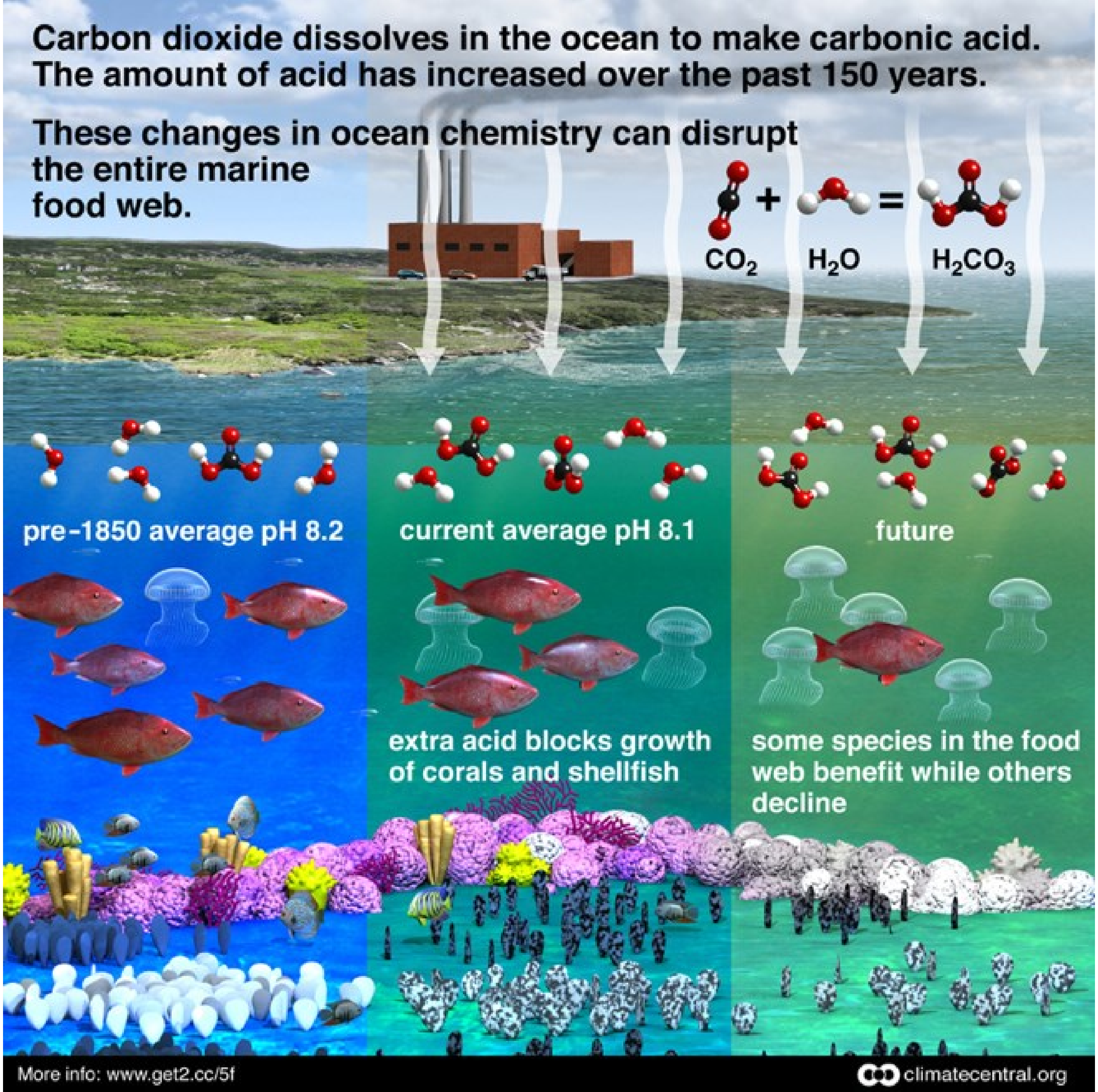
- invasions from populations living in warmer habitats, competing with local populations
- Decreases in cold adapted species

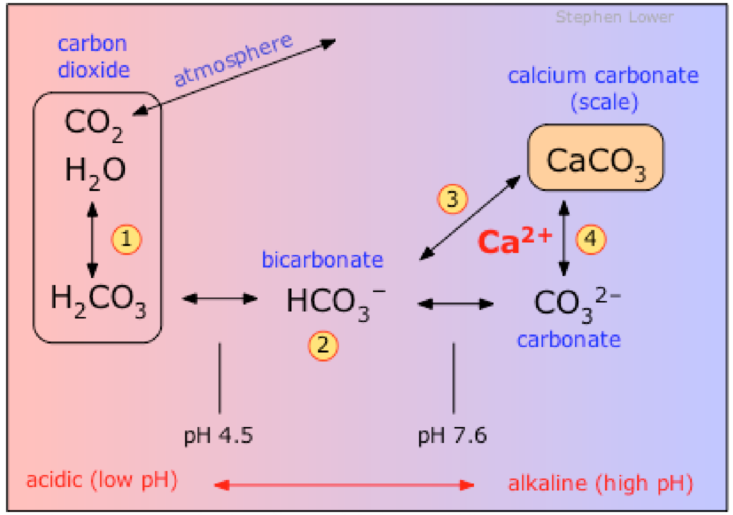
Ocean Acidification



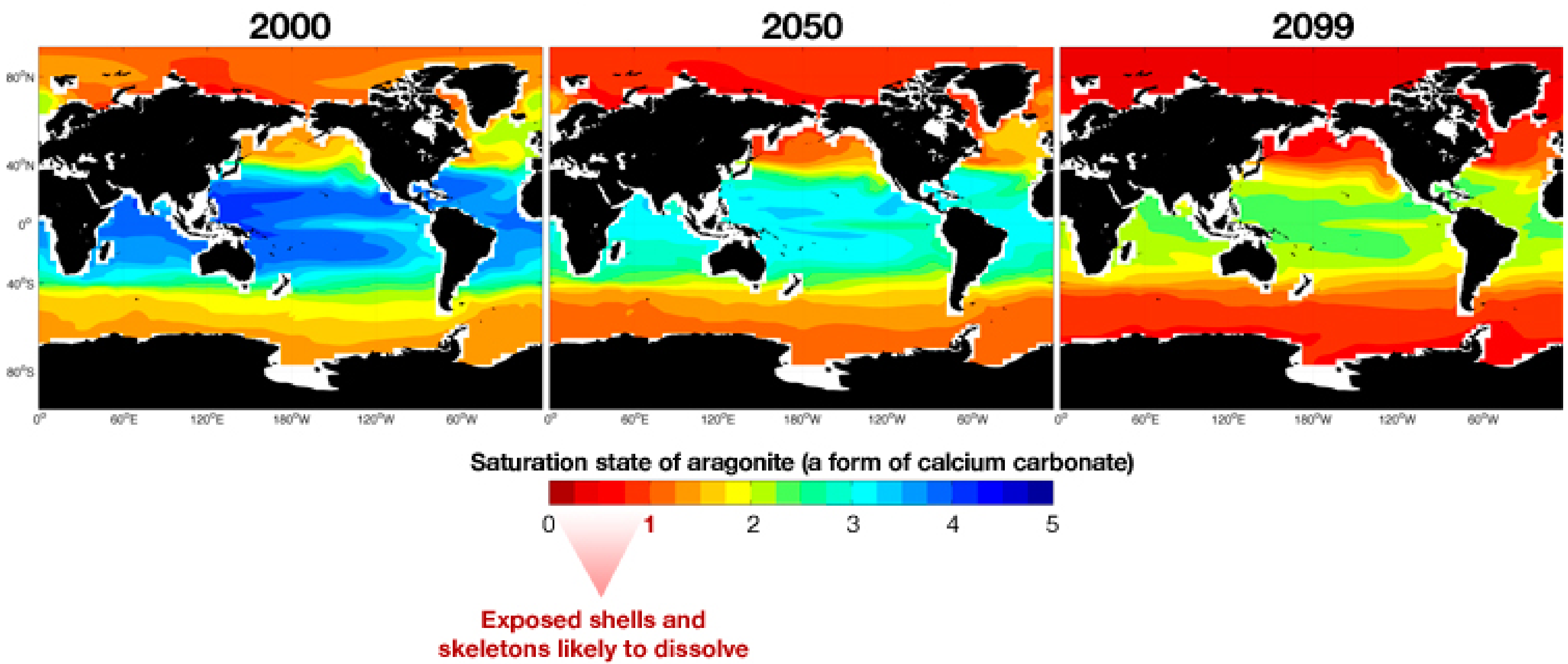


Atmospheric CO2 (ppmv)
Seawater pCO2 (µatm)
Seawater pH
400
375
350
325
300
275
CO2
8.38
8.33
8.28
8.23
8.18
8.13
8.08
8.03
pH
1940
1950
1960
1970
1980
1990
2000
2010
Year


Carbon dioxide dissolves in the ocean to make carbonic acid. The amount of acid has increased over the past 150 years.
These changes in ocean chemistry can disrupt the entire marine food web.
CO2
H2O
H2CO3
pre-1850 average pH 8.2
current average pH 8.1
future
extra acid blocks growth of corals and shellfish
some species in the food web benefit while others decline
More info: www.get2.cc/5f
climatecentral.org



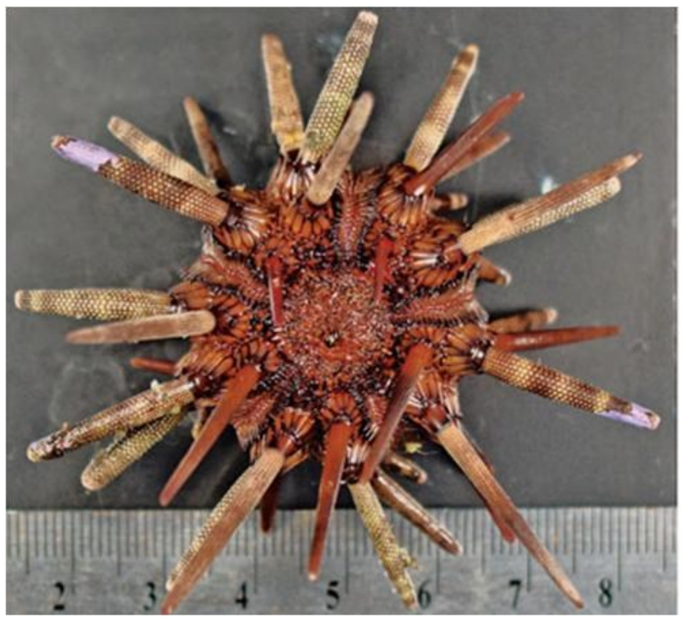
Carbonate levels predicted to drop as ocean acidifies



FLORIDA KEYS

1980

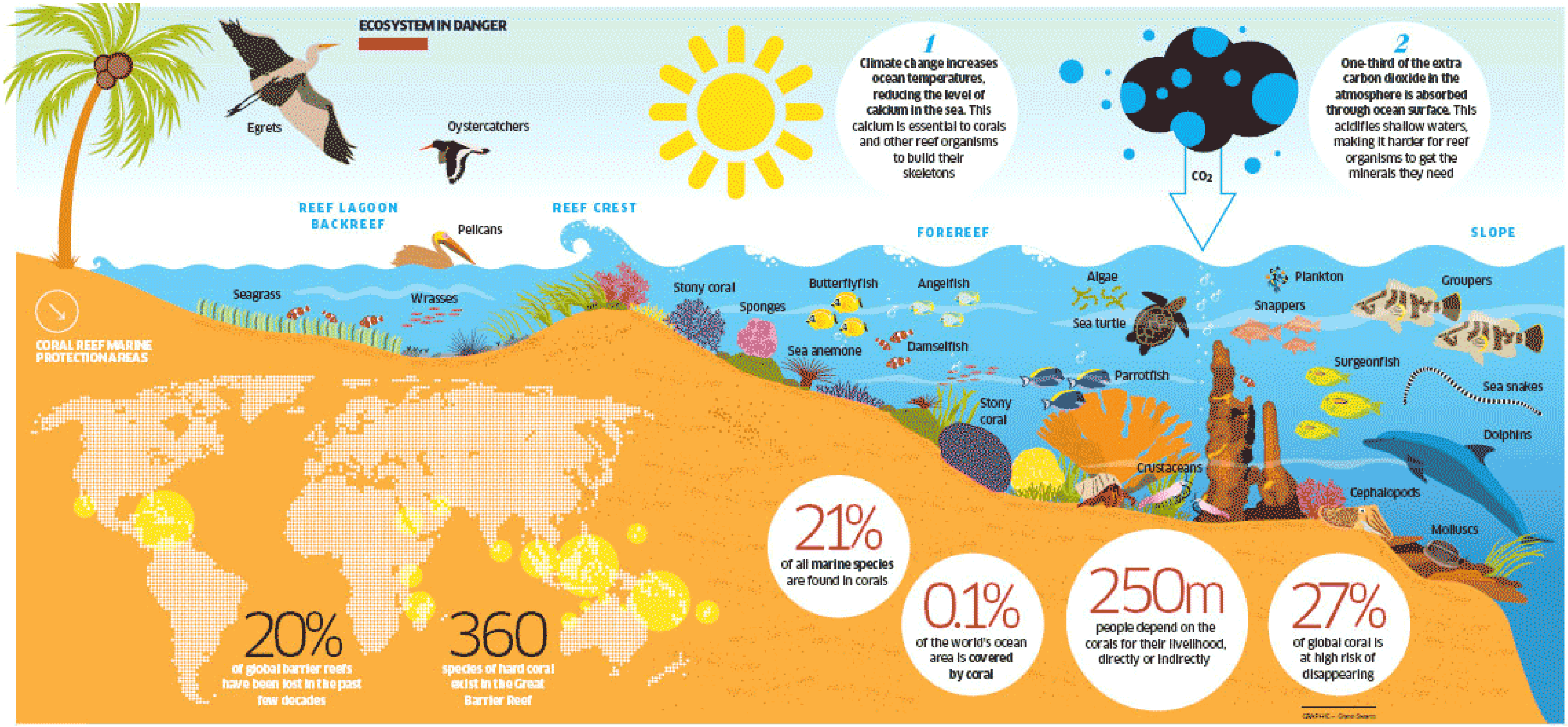
2010



Pencil urchin under normal CO2

Pencil urchin under high CO2 (2850 ppm) showing dissolution of spines

Major impact on coral reefs – a major source of biodiversity



BIOMASS:

-> only renewable organic source

-> fixes CO2 with Photosynthesis

$$CO_2 + H_2O + \text{light energy} \rightarrow [CH_2O] + O_2$$

Renewable and Sustainable Energy Reviews 62 (2016) 134–163

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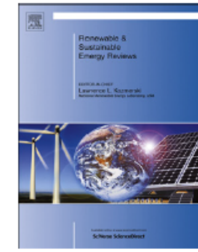


Can photosynthesis enable a global transition from fossil fuels to solar fuels, to mitigate climate change and fuel-supply limitations?

CrossMark

Andrew K. Ringsmuth [a,b,1], Michael J. Landsberg [a,2], Ben Hankamer [a,*]

[a] *The University of Queensland, Institute for Molecular Bioscience, St Lucia, Queensland 4072, Australia*
[b] *The University of Queensland, ARC Centre of Excellence for Engineered Quantum Systems, St Lucia, Queensland 4072, Australia*

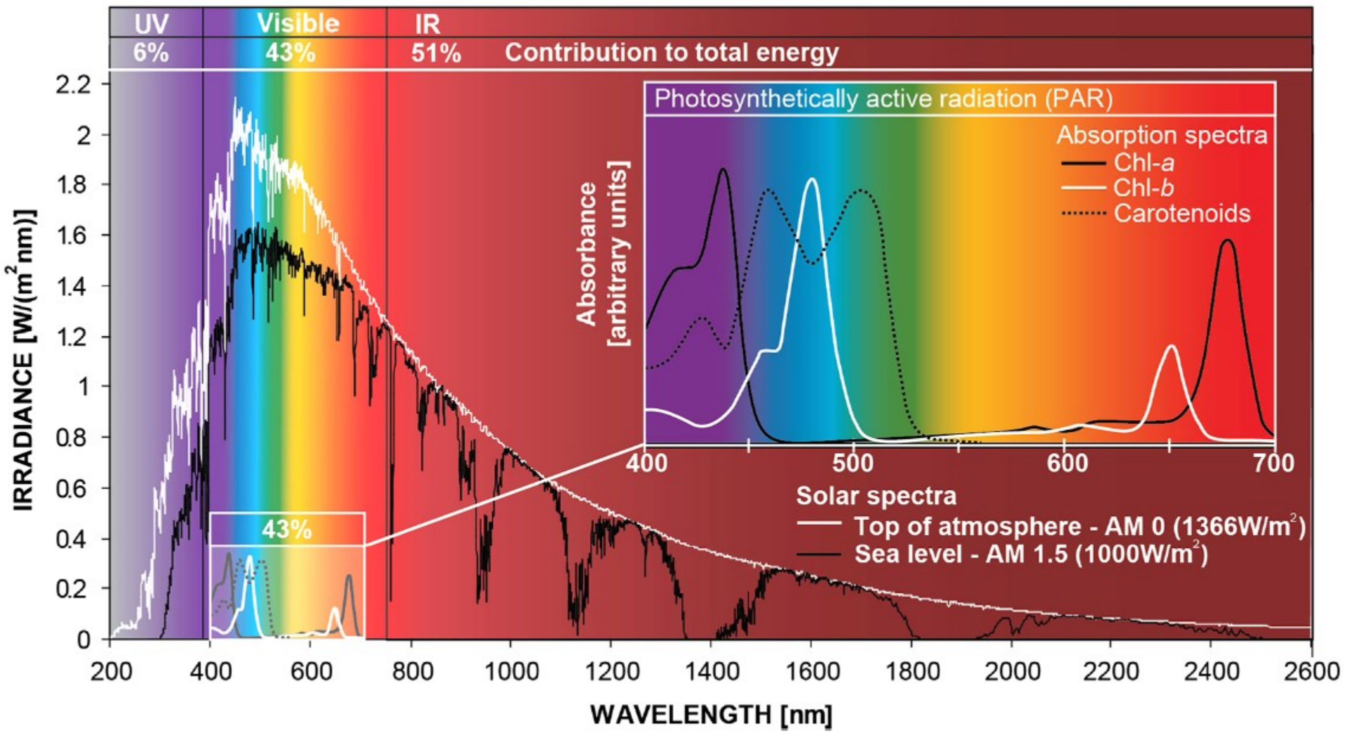
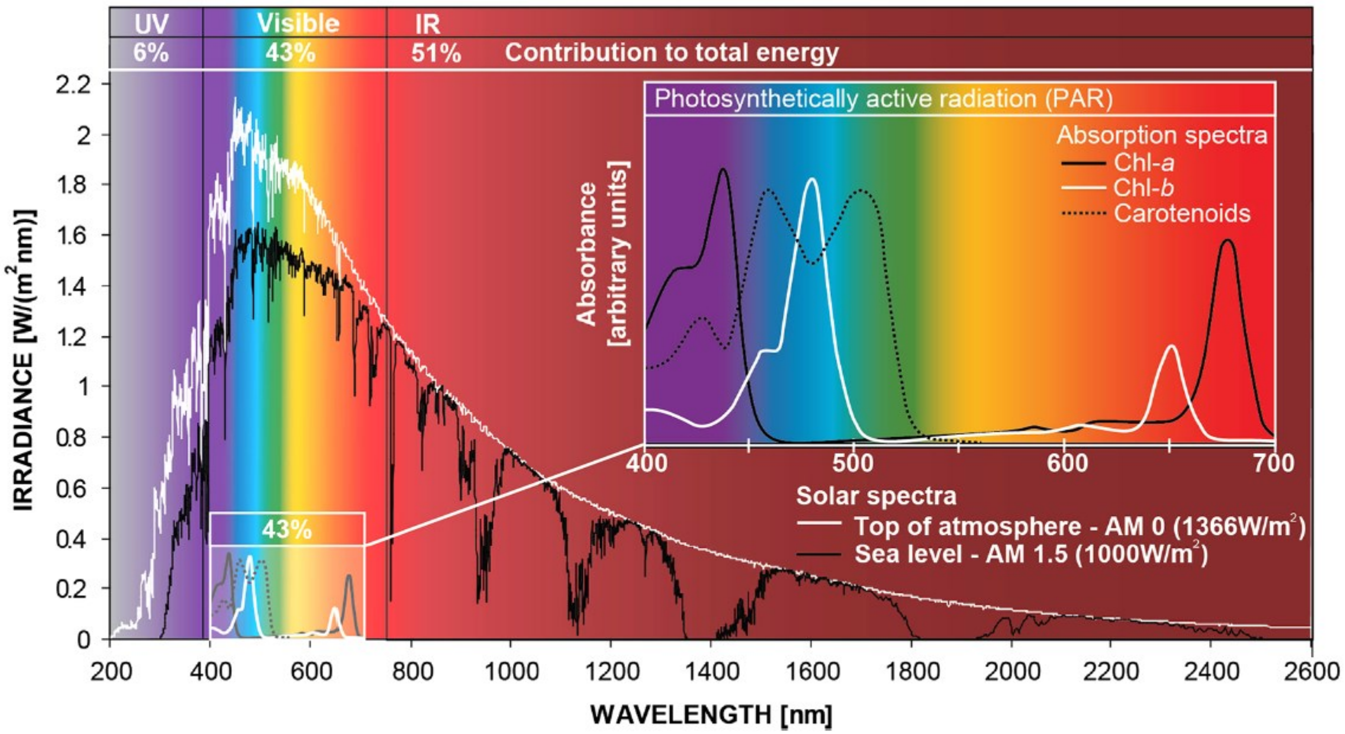


Fig. 1. Terrestrial solar irradiance and photosynthetic absorption spectra. AM0 and standard solar spectra (see Section 3.2) are shown. Atmospheric absorption bands are visible in the AM1.5 spectrum. Inset shows in vivo absorption spectra for pigments from higher plants and green algae. Inset adapted from [30]. Overall figure adapted from [30,31].

PAR – Photosynthetic Active Radiation (400-700 nm) is 43% of the active solar radiation

Large IR fraction
6% of UV



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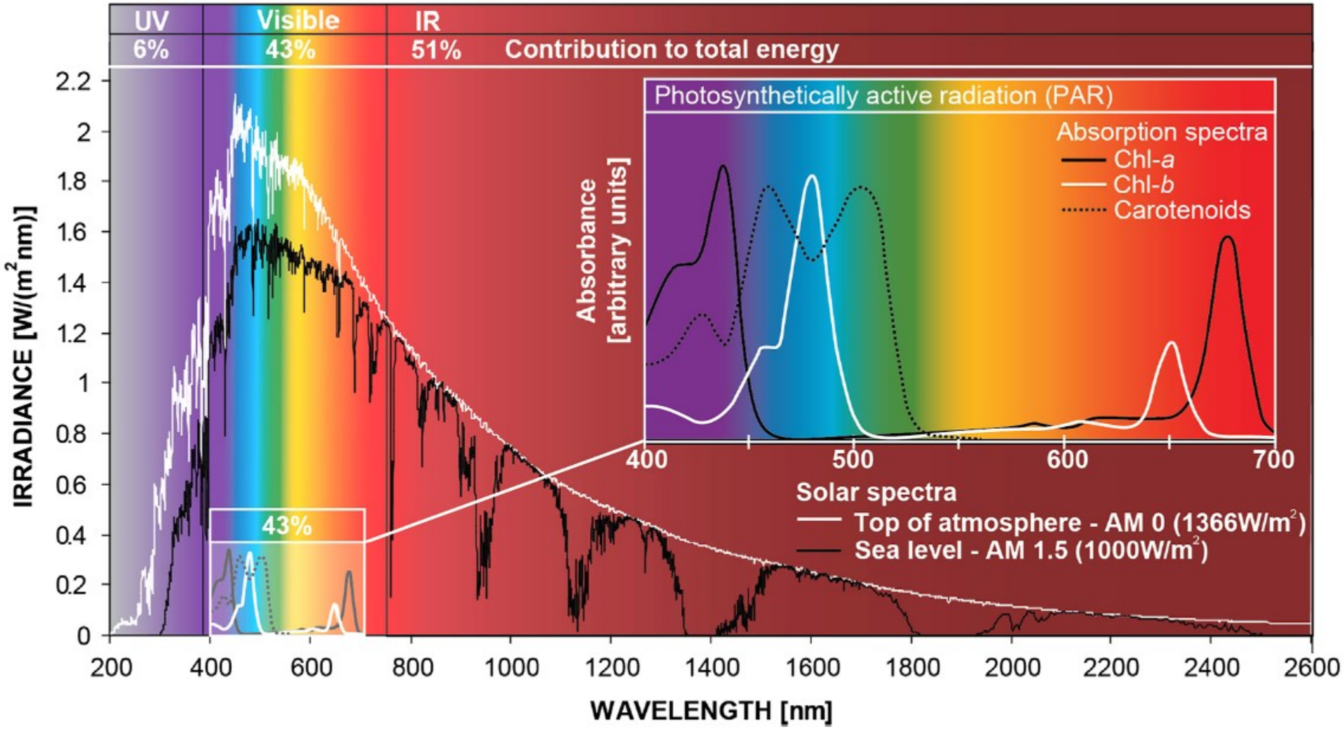
Total energy from sun 5490 Zj energy (zetta Joules = 10^{21} Joules)

Vs. 0.503 ZJ used every year by the global economy (11 000 more)



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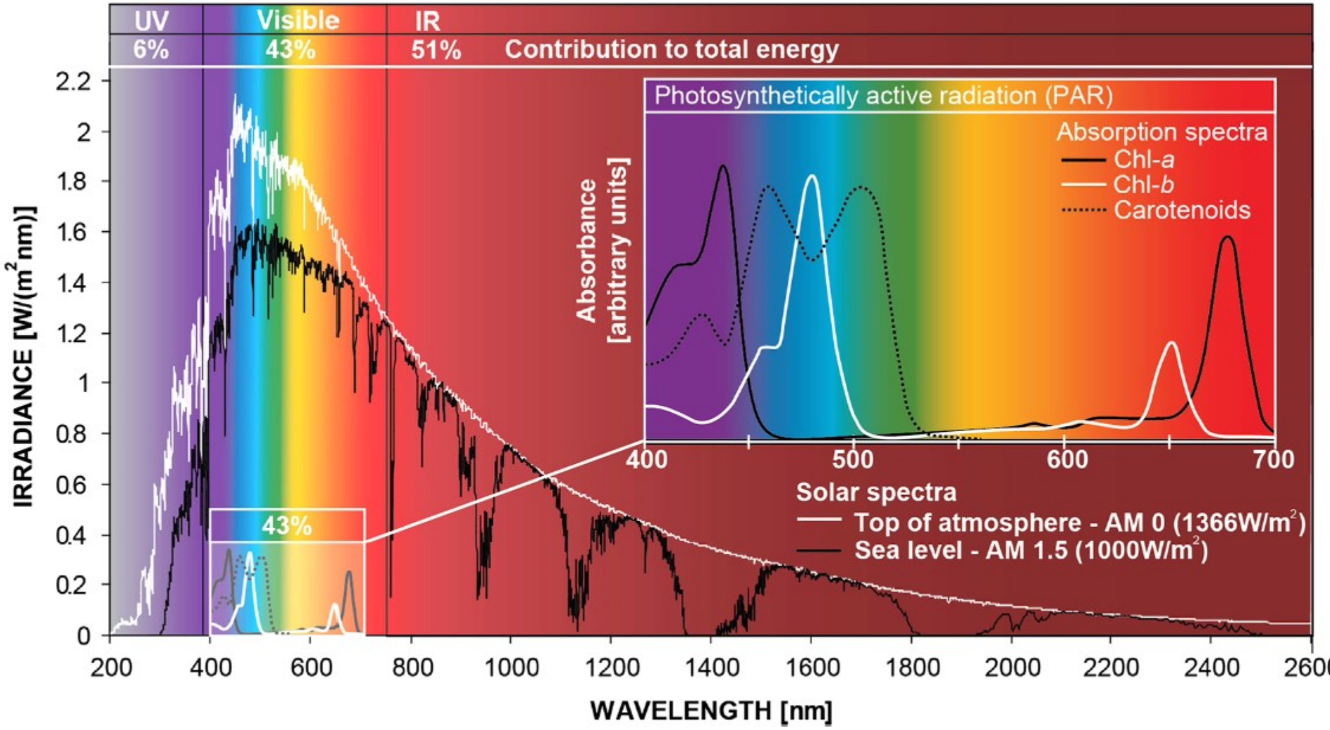
Atmosphere absorbs part of the radiation – leaving 3020 ZJ / year

80 % reach the oceans
20% reach the land



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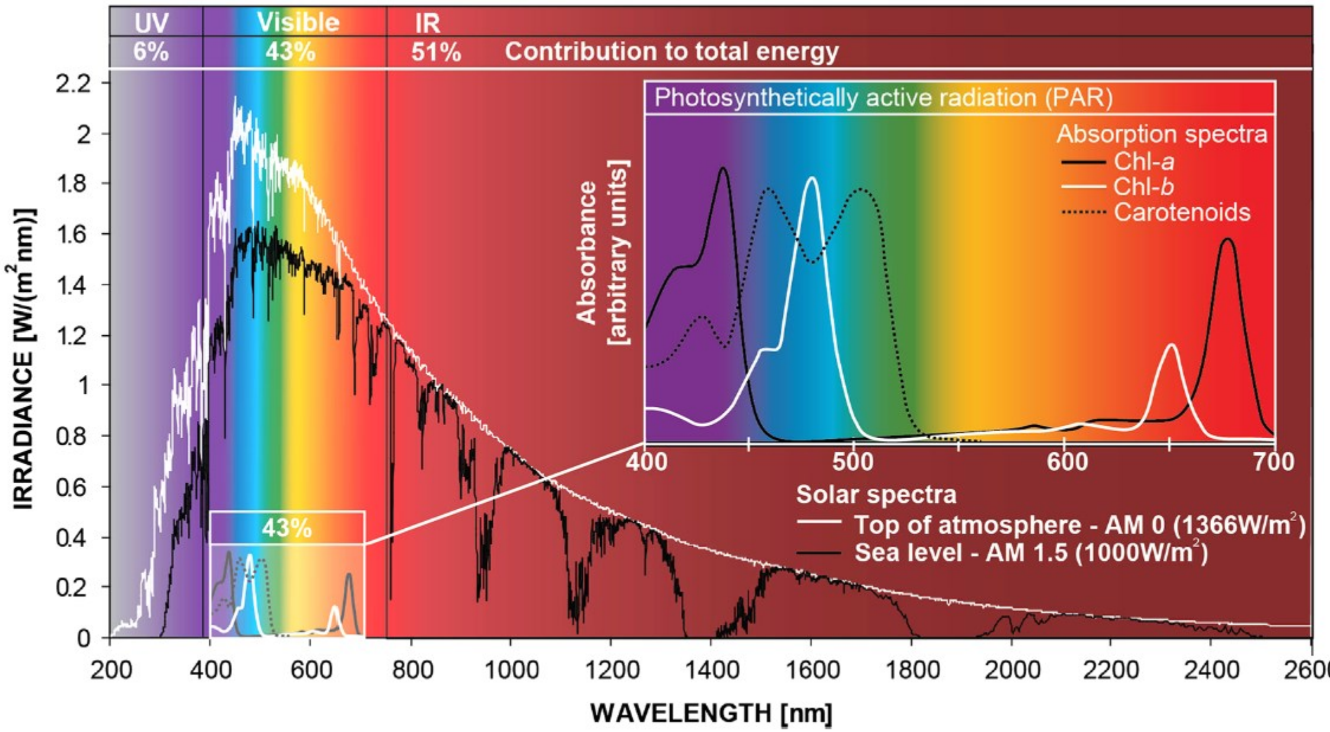
Average insolation is 188 W m^{-2}

There is a huge geographical variability: between 12 to 405 W m^{-2}



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Average insolation is 188 W m^{-2}

Considering efficiency in conversion (e.g. 10% for PV) this is

≈ 1-2 order of magnitude smaller than energy density from a thermal power plant

This is also temporally intermittent

Challenge: store solar energy in a stable, concentrated form -> Chemical Fuels

Is Biology capable of producing these amounts of energy?

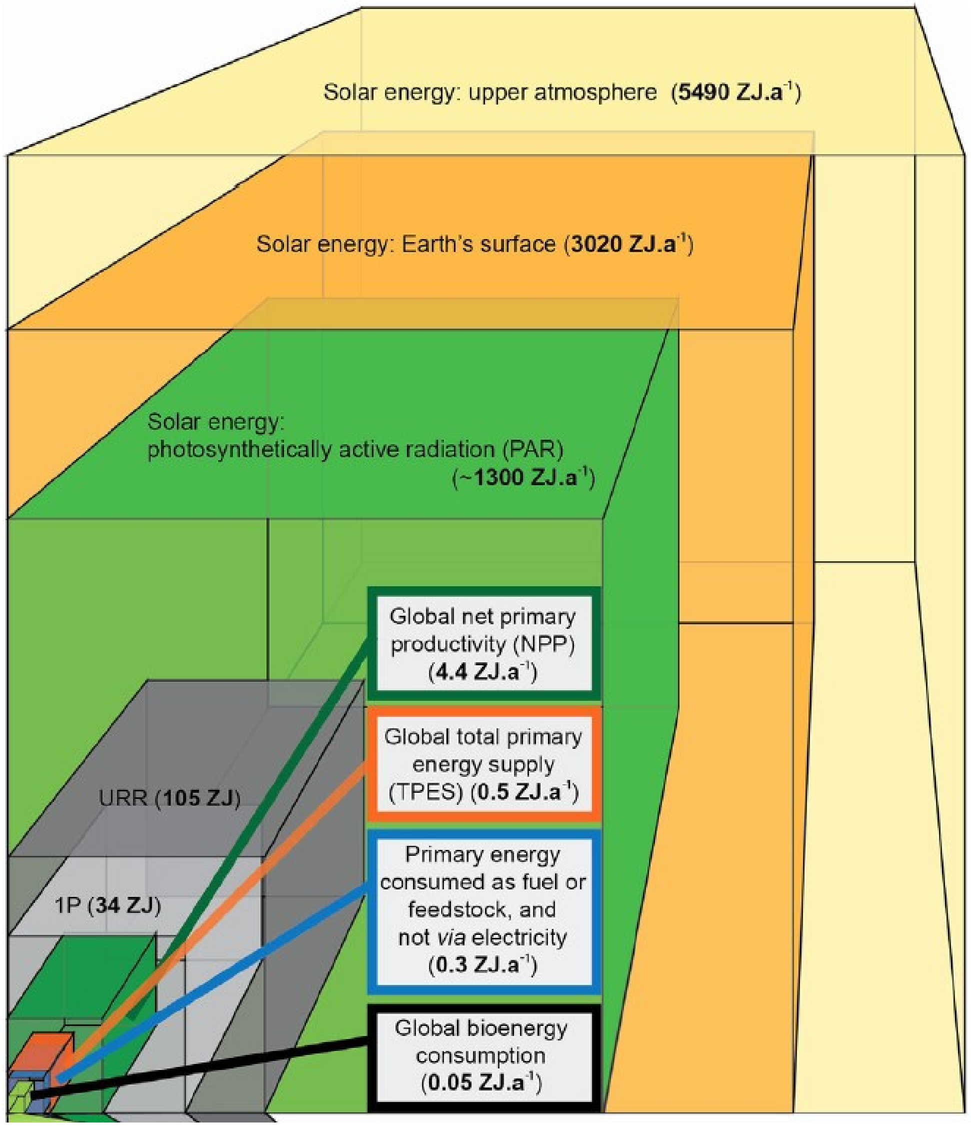
Primary production = synthesis of new biomass from inorganic precursors

Photosynthesis or Chemosynthesis

GROSS PRIMARY PRODUCTION (GPP) = total photosynthetic primary production

NET PRIMARY PRODUCTION (NPP) = GPP – autotrophic respiration =
biomass available to other organisms

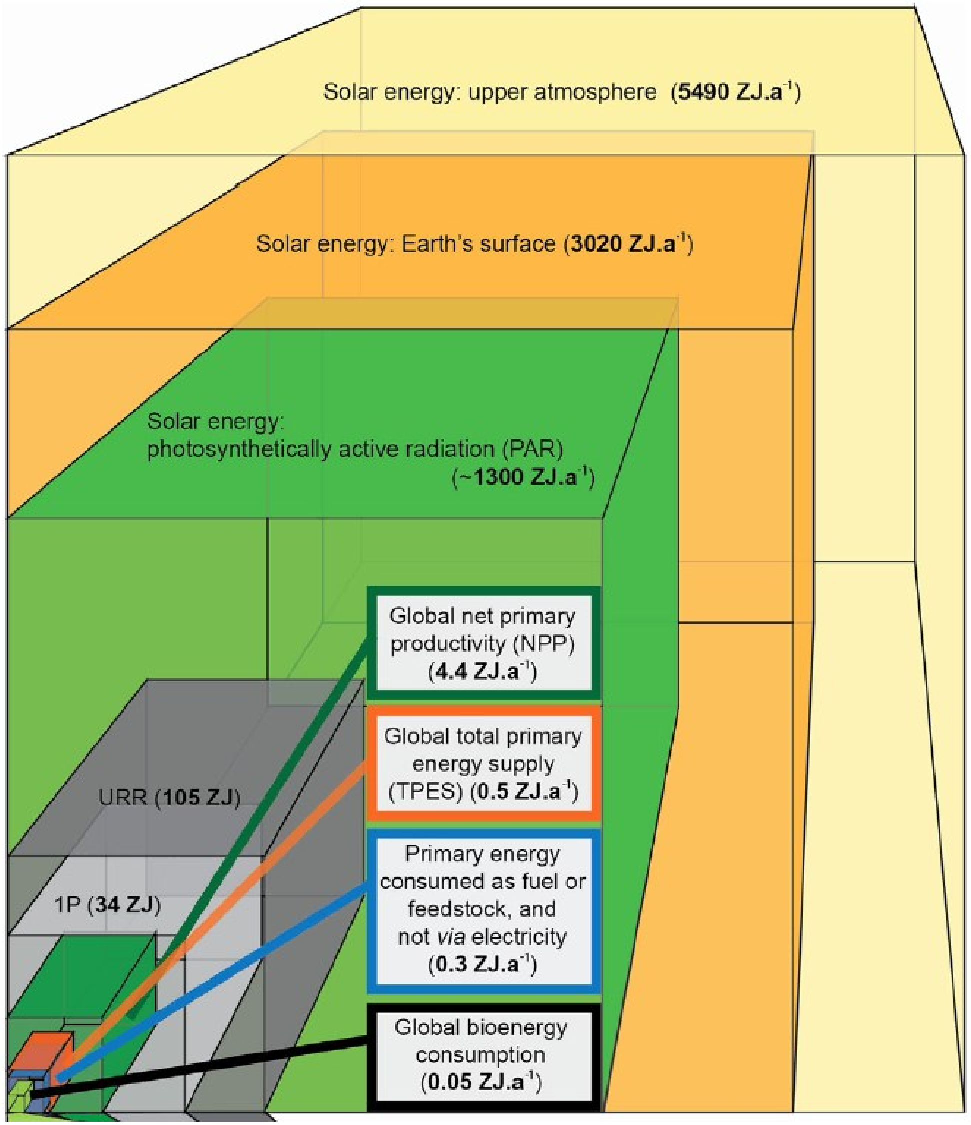
3020 ZJ / year available to photosynthesis
Only 43% (1300 ZJ/y) is PAR radiation



Terrestrial NPP = 53-59 GtC / year

1 Kg biomass = 0.45 Kg C = 17.5 MJ

2.1 ZJ



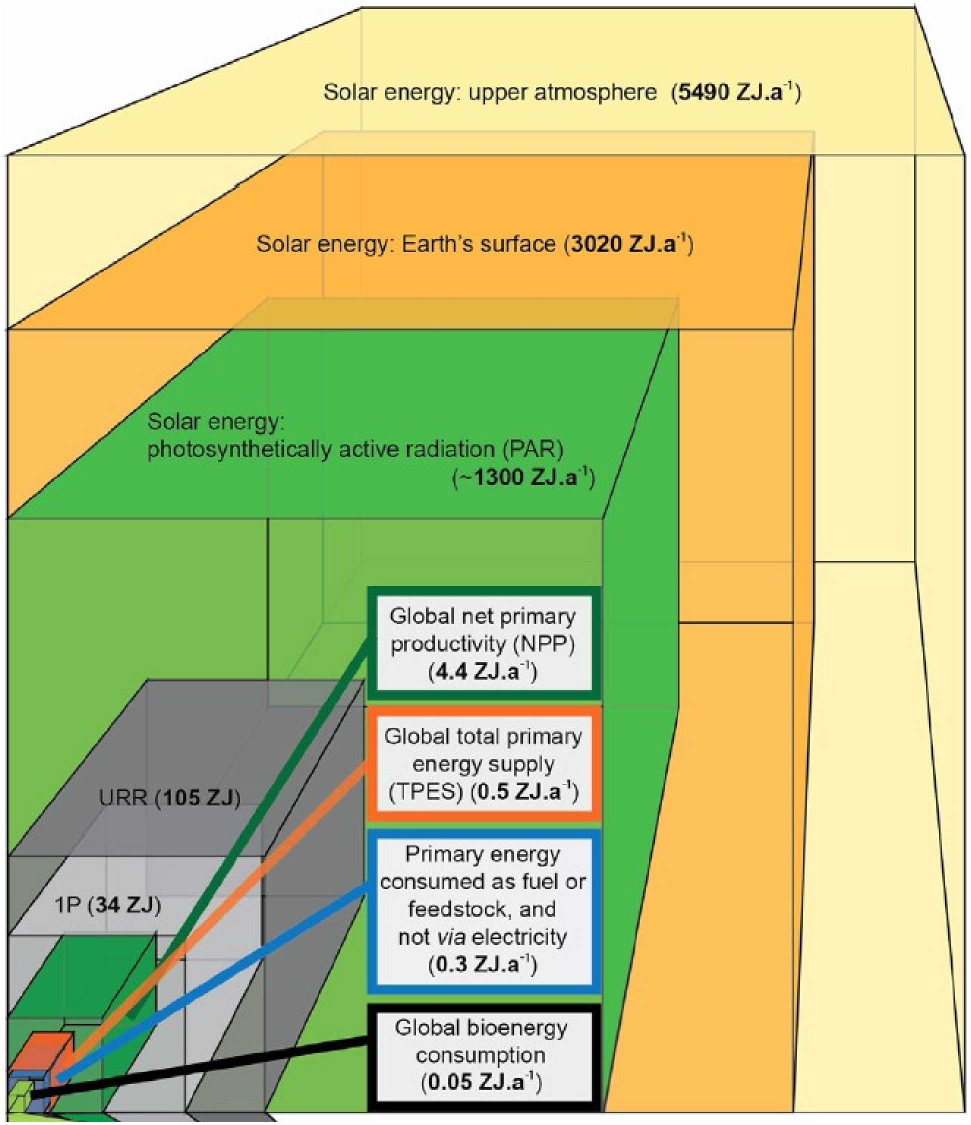
water NPP ≈ 55 GtC / year

1 Kg biomass = 0.47 Kg C = 20 MJ

2.3 ZJ

total NPP ≈ 4.4 ZJ / year

9 times the 0.503 ZJ used every year by the global economy



Impact also on C balance:

Most of the biomass is used by heterotrophic organisms

But 4.8 GtC / year are long term sinks

(48% of anthropogenic emissions)

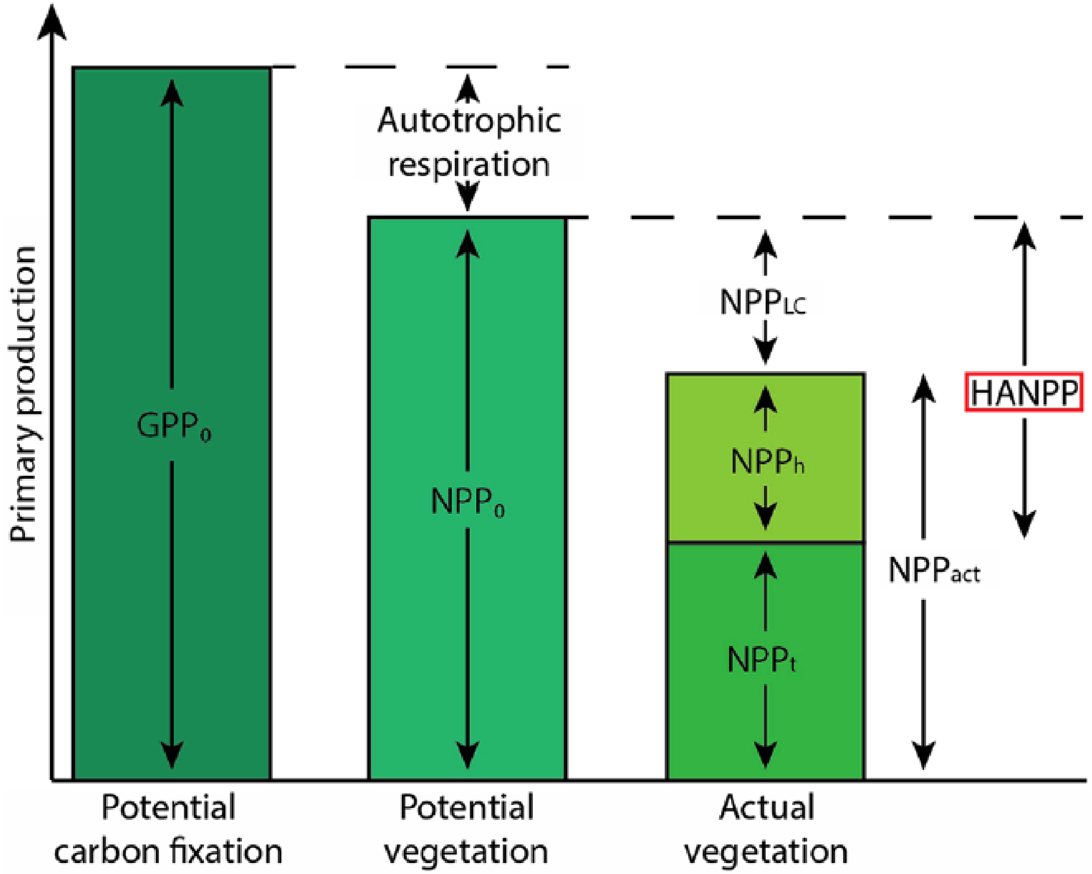
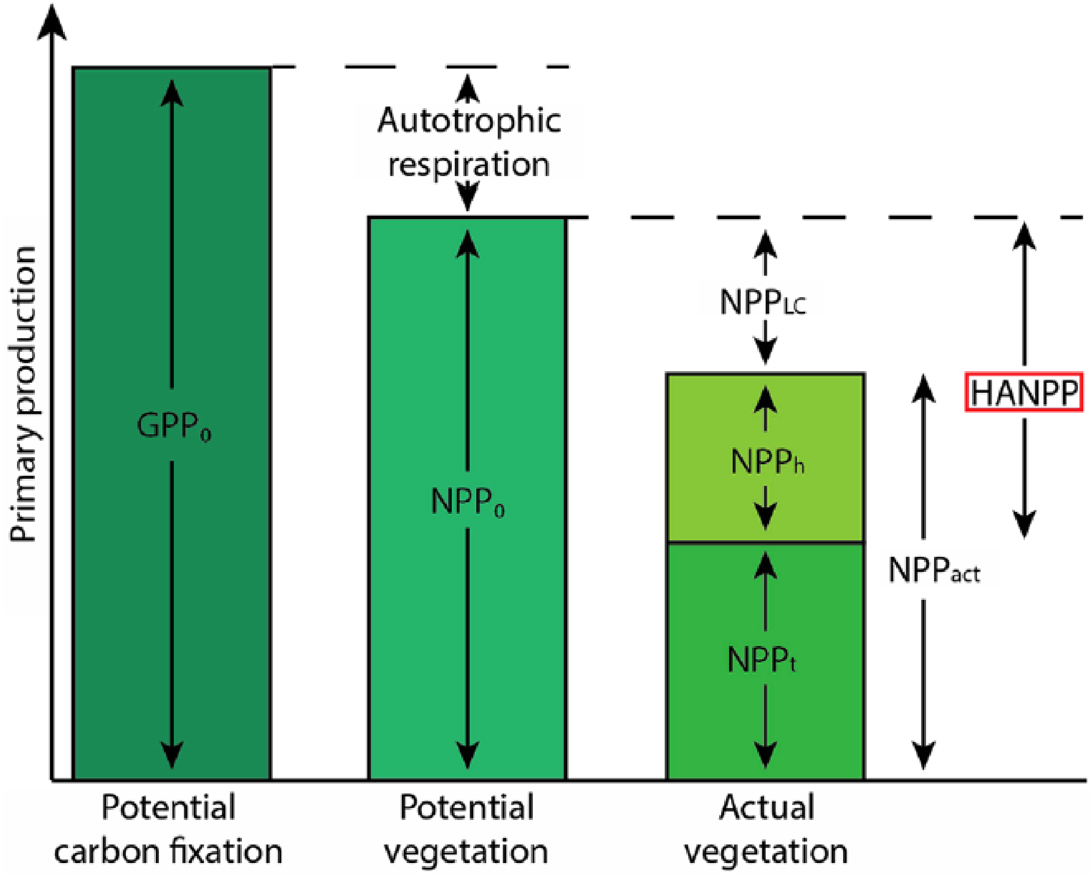


Fig. 3. The standard definition of human appropriation of net primary production (HANPP), graphically represented. Terms are defined in Section 2.6.1. Figure adapted from [42].

Loss in NPP because of human activity (land use changes)

Extraction / destruction of NPP for human purposes (biomass harvest / grazing)

HANPP = human appropriation of net primary production



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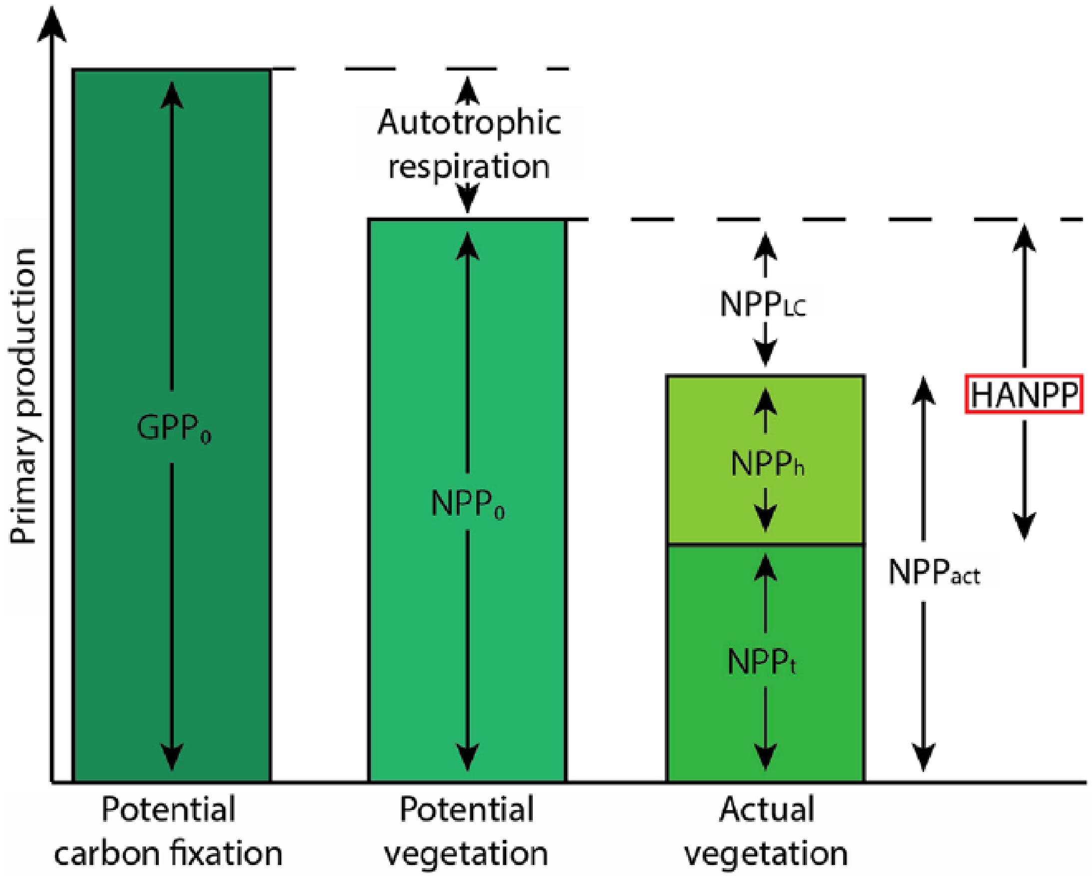
Terrestrial HANPP estimated to be 28% of NPP

0.32 KJ energy (64% of global energy demand)

aquatic HANPP estimated to be 8% of NPP (to support fish harvest)

HANPP = human appropriation of net primary production



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High HANPP are a threat for biodiversity and sustainability

e.g. other species need NPP

A sustainable world should use 60% less of HANPP

Increasing HANPP is not the answer for improving our energy demand

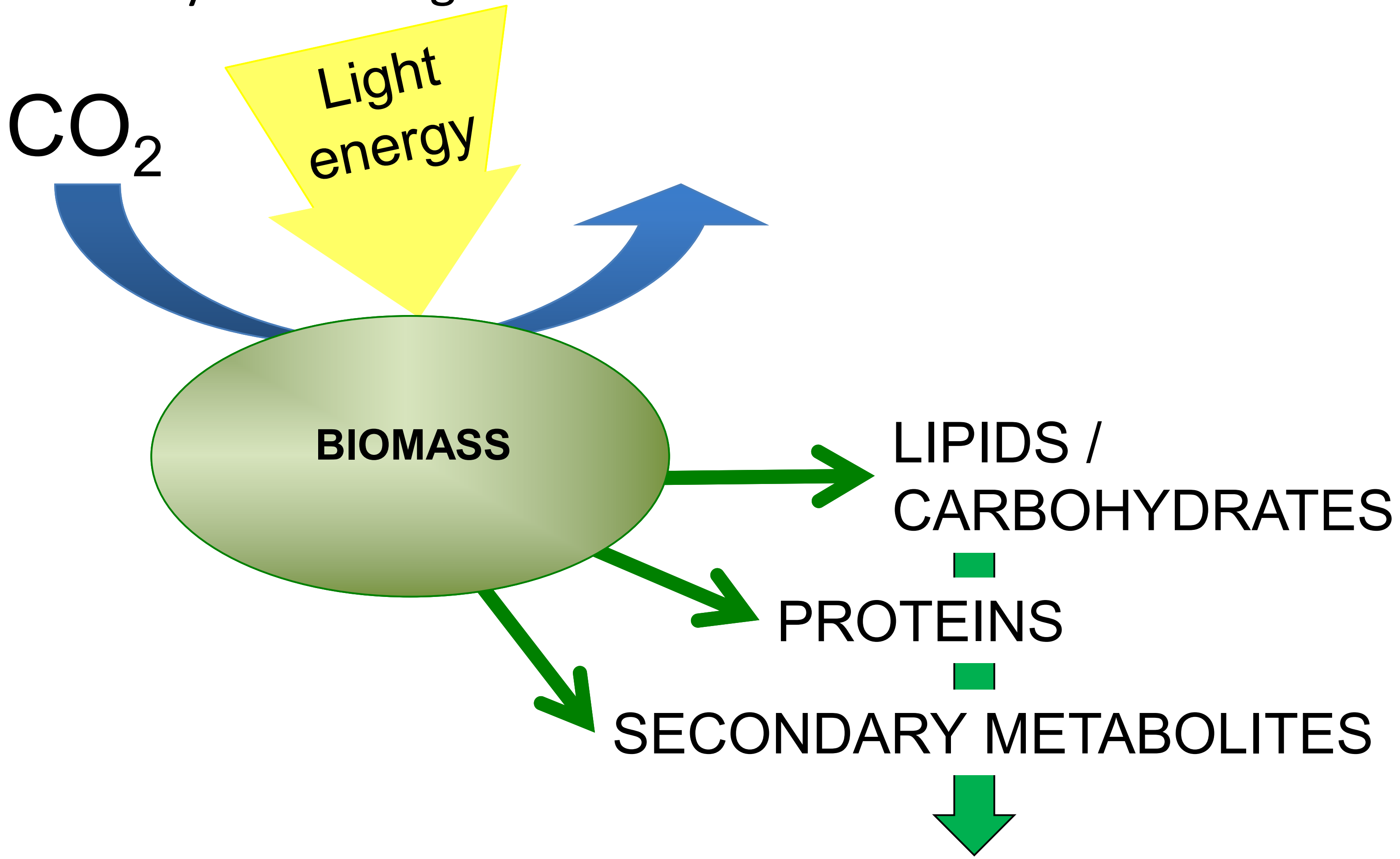
Food demand will increase in next decades. There will be no likely no increase in NPP available for energy purposes.

Need for

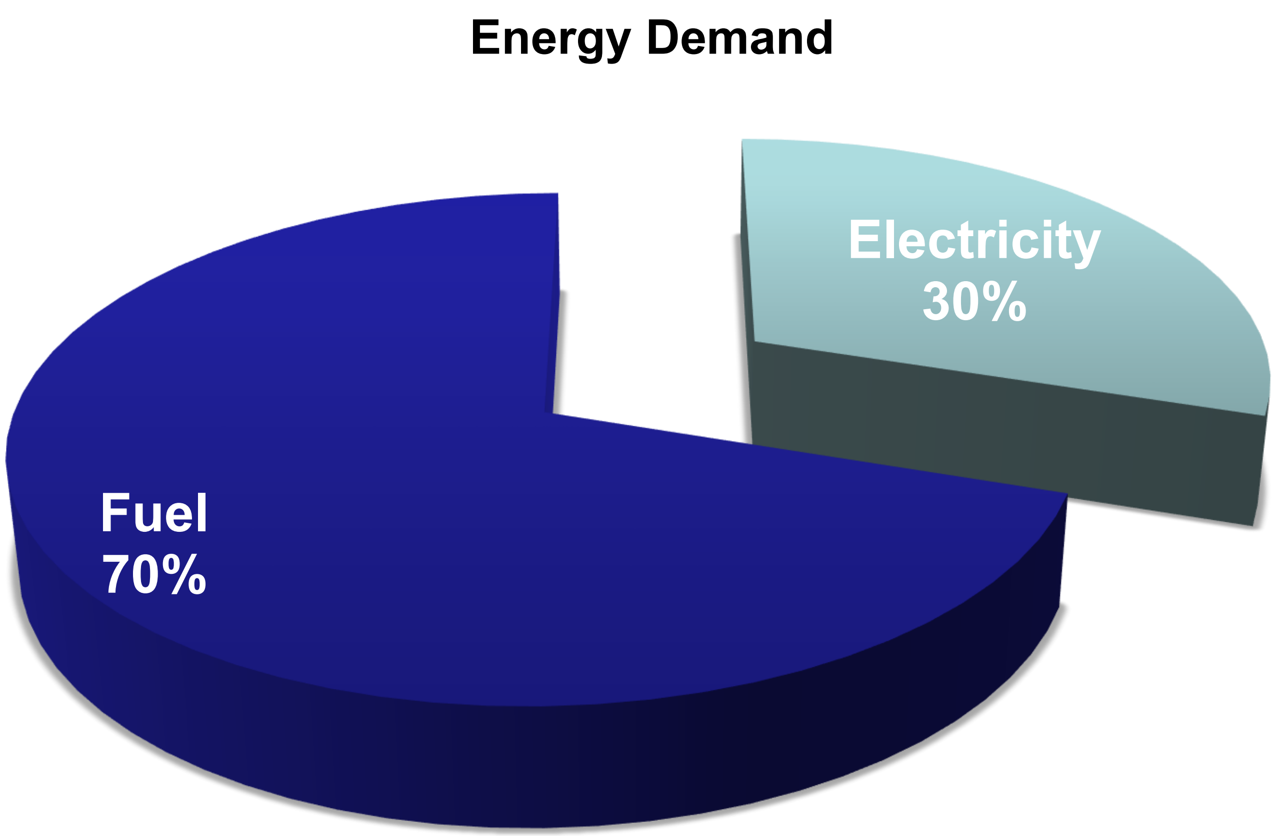
- Improved efficiency in biomass use
- Increase earth photosynthetic productivity, (e.g. use unproductive areas)

The challenge of reducing CO_2 emissions

Photosynthetic organisms can contribute to this reduction

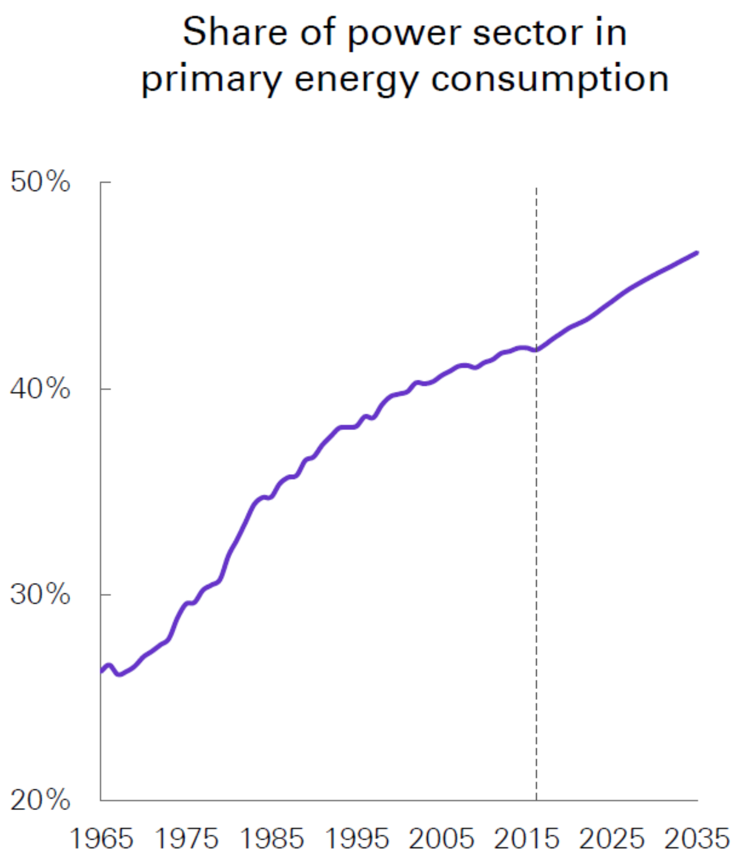


Can be used to produce FUELS, CHEMICALS, PLASTICS …

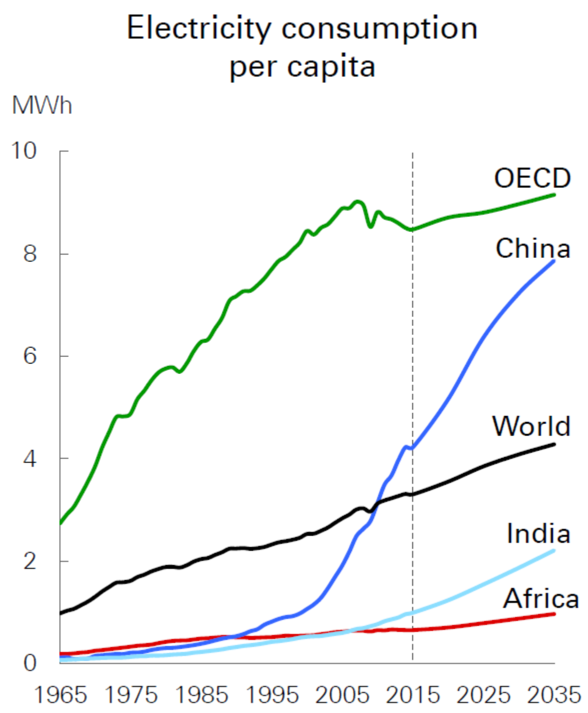

Energy Demand
Electricity
30%
Fuel
70%

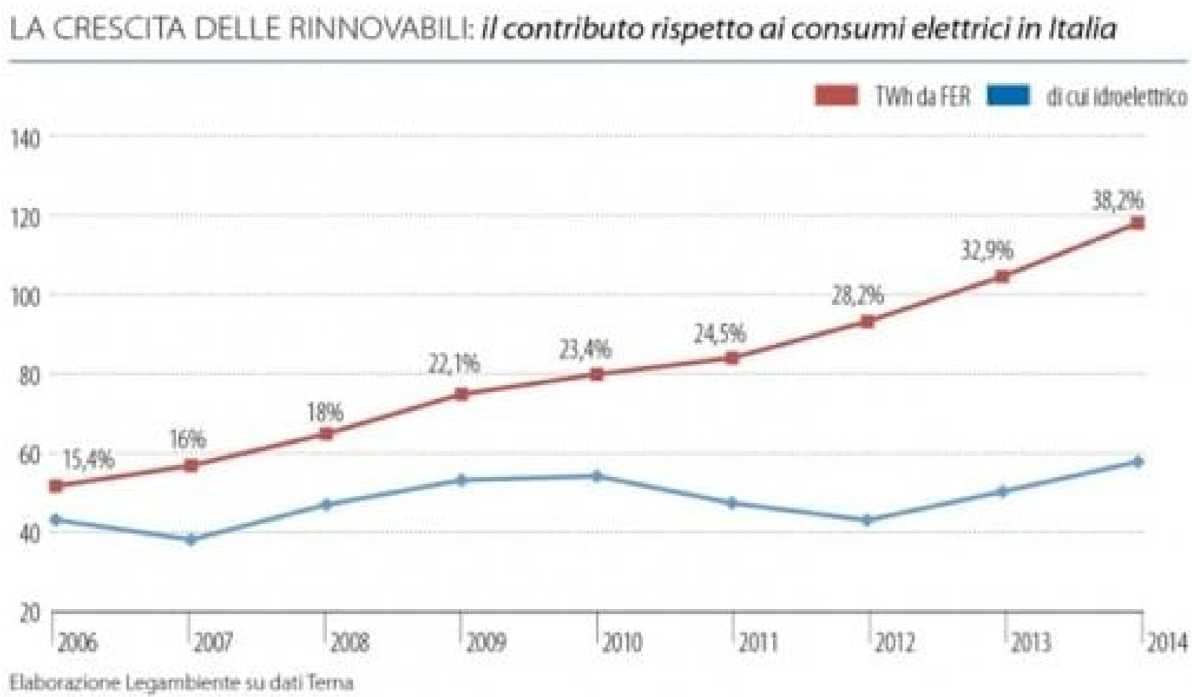
RENEWABLE ENERGY SOURCES

Energy Demand



Electricity consumptions increases





Electric power from renewable sources is a large portion of current production. There are issues:

- Still expensive
- Issues with constant supply

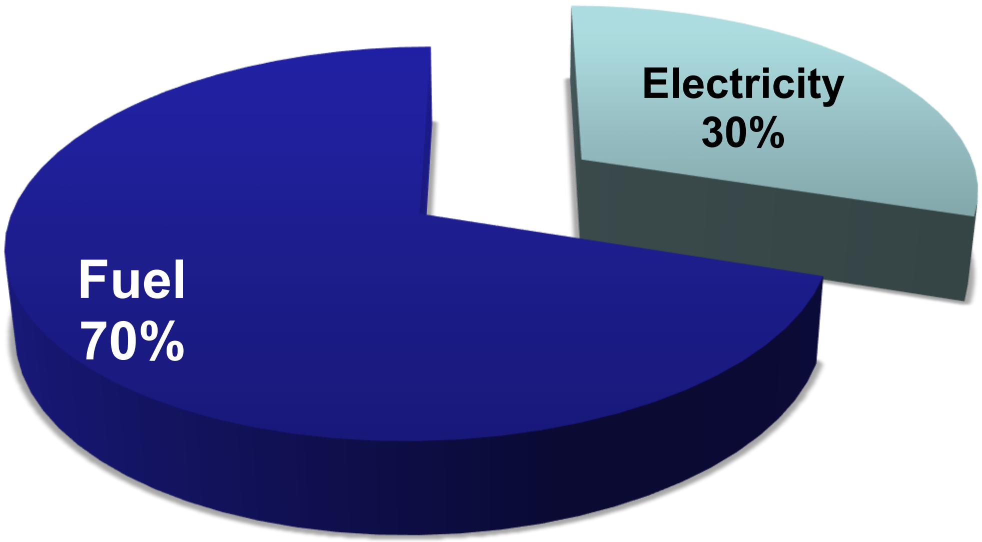
RENEWABLE ENERGY SOURCES

Different renewable sources :

- Photovoltaic
- Wind
- Hydroelectric
- Biodiesel
- Bioethanol
- Geothermic

Energy Demand

Liquid fuels

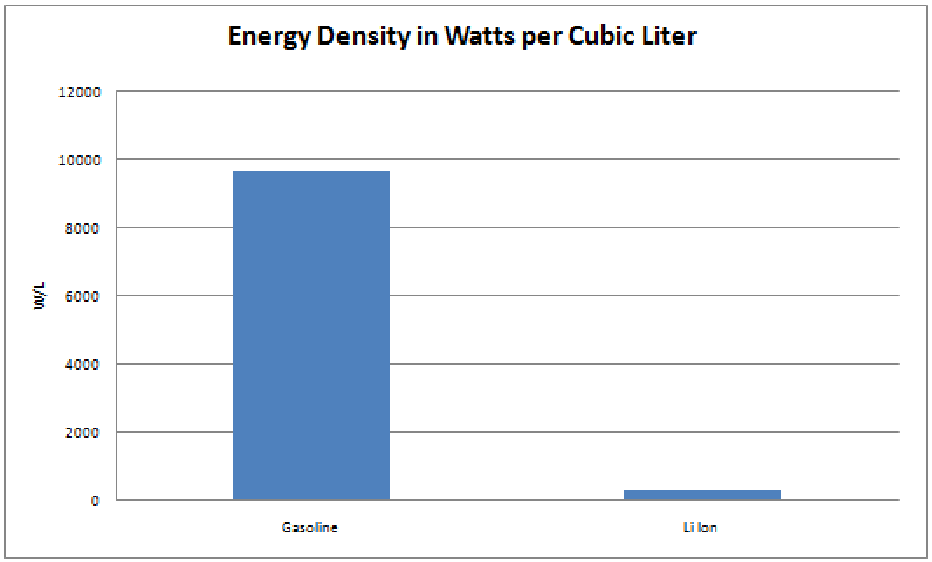


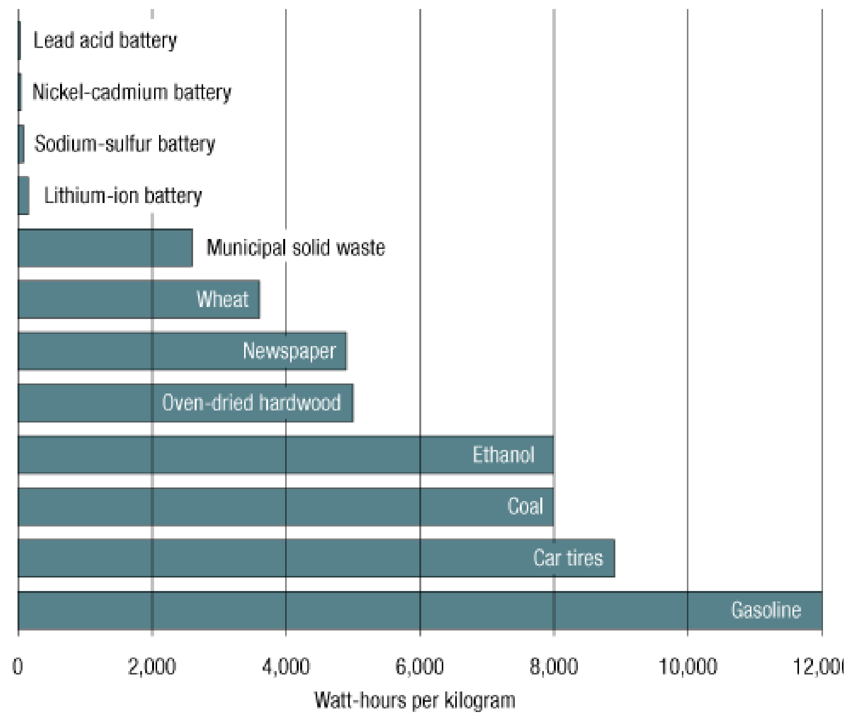
Why liquid fuels are so important for transportation?

1 l of gasoline energy of ≈ 7000 cellphone batteries

Why liquid fuels are so important for transportation?

Energy density is much higher for gasoline





Using Electric power for transportation is difficult with present technology

Impossible for aviation

The lithium-ion battery pack in a Tesla Roadster weighs about 1,000 pounds (453.6 kg). That's a lot of weight to carry and it can greatly reduce the car's range. However, the designers of the Roadster have offset this battery weight with a light frame and body panels. The entire car only weighs 2,690 pounds (1220.2 kg) -- not terribly heavy when you consider that more than a third of that weight is battery.

Airbus' two-seat electric plane could only go a maximum speed of about 136 miles per hour. A solar-powered plane that completed an around-the-world journey this summer had an average airspeed of 47 miles per hour. The plane, called Solar Impulse 2, had more than 17,000 solar cells that powered four electric motors.

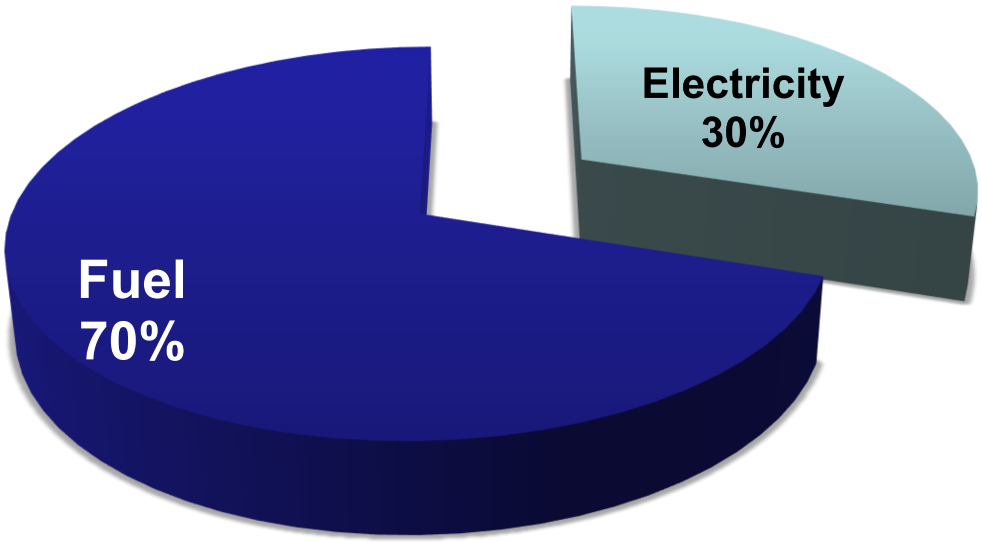
RENEWABLE ENERGY SOURCES

Different renewable sources :

- Photovoltaic
- Wind
- Hydroelectric
- Biodiesel
- Bioethanol
- Geothermic

Energy Demand

Liquid fuels



BIOFUELS

1^{st} Generation Biofuels : crop grains / seeds to extract starch or oil

2^{nd} Generation: non food crops

3^{rd} Generation: Algae

Table 1. Comparison of biofuel sources

Biofuel generation	First (crop species)	Second (grasses and trees)	Third (algae)	Refs
Primary products	Bioethanol Biodiesel	Bioethanol Solid fuel Hydrogen gas	Biodiesel Hydrogen gas	
Secondary products	Biomethane Distillers grain Animal feed		Bioethanol Biomethane Glycerol Animal feed Pigments	[57,71]
Example species	Maize (*Zea mays*) Oil palm (*Elaeis guineensis*) Sugarcane (*Saccharum* spp.)	Poplar (*Populus* spp.) Miscanthus (*Miscanthus* spp.)	*Dunaliella* spp. *Nannochloropsis* spp. *Botryococcus* spp.	
Primary product cost (US$)/L biofuel (current)	0.45–0.55	0.80–1.20	1.50–2.50	[5,72]
Primary product cost (US$)/L biofuel (potential)	0.40–0.50	0.55–0.70	0.50–1.00	[5,72]
Potential fuel yield (L biofuel/ha/y)	200–7500	5000–12 000	50 000–120 000	[5,73]
Land requirement	High-quality agricultural land	Marginal land	Low-quality land	
Other requirements	Freshwater source	Extensive processing Freshwater source	High sunlight irradiance Close proximity to sea water CO_2 source	

Biofuels
currently 3% of road transport fuel supply

Will reach 9 % by 2050 (Alternative energies for transport, Shell)

Biofuels are in general limited by feedstock

Feedstock price now account for 45-70% of total production cost (www.iea.org)

2. Bioetanolo

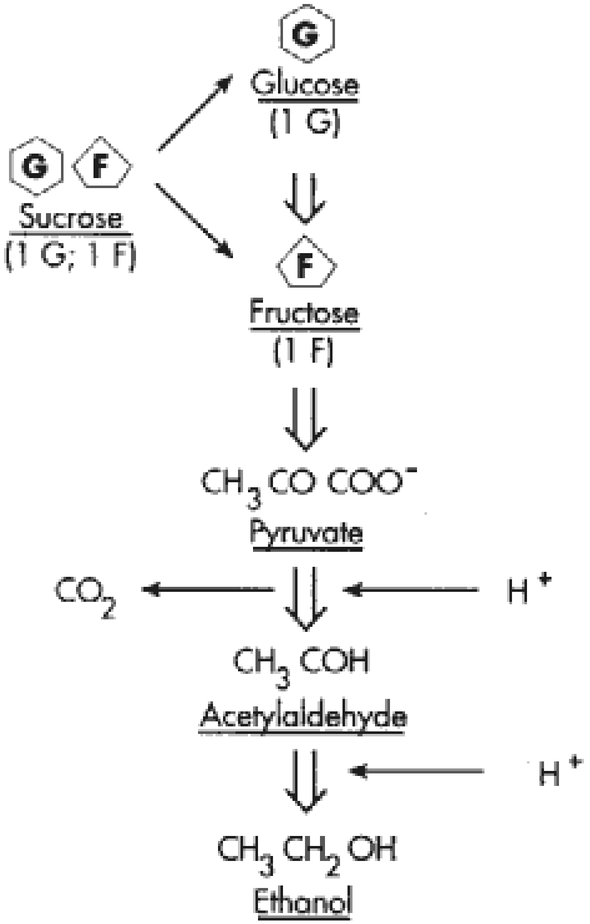
BIO-ETHANOL

Ethanol production from fermentation

-The oldest biotechnology

- wine making around 5000 BC in modern Iran

- in Egypt 3000 BC strains similar to Saccaromyces cerevisiae were employed for wine and beer making.



BIO ETHANOL

Distillation yields 95% Ethanol
Highly miscible with water

Inflammable

-In 1905 ethanol was emerging as the fuel of choice for automobile (early Ford model could use gasoline, ethanol and a mixture of them)

-Gasoline was later chosen because of price competition.

-Before WWI industrial gasoline production increased
before that oil was employed for kerosene production, exploited for lighting domestic homes (overtaken by electricity)

- Development driven by increase of US oil production

BIO ETHANOL

Ethanol used mixed with Gasoline

-suitable for internal combustion engines

PROs -> high octane number
high heats of vaporization

Efficiency advantages over gasoline

CONs -> Lower energy content (35% less)
Lower vapor pressure (difficult for cold starts)

Can be used in Blends – up to 20% withouth modifications

Commercial names E10
E85 - > requires FFV (Flexible Fuel Veichles)

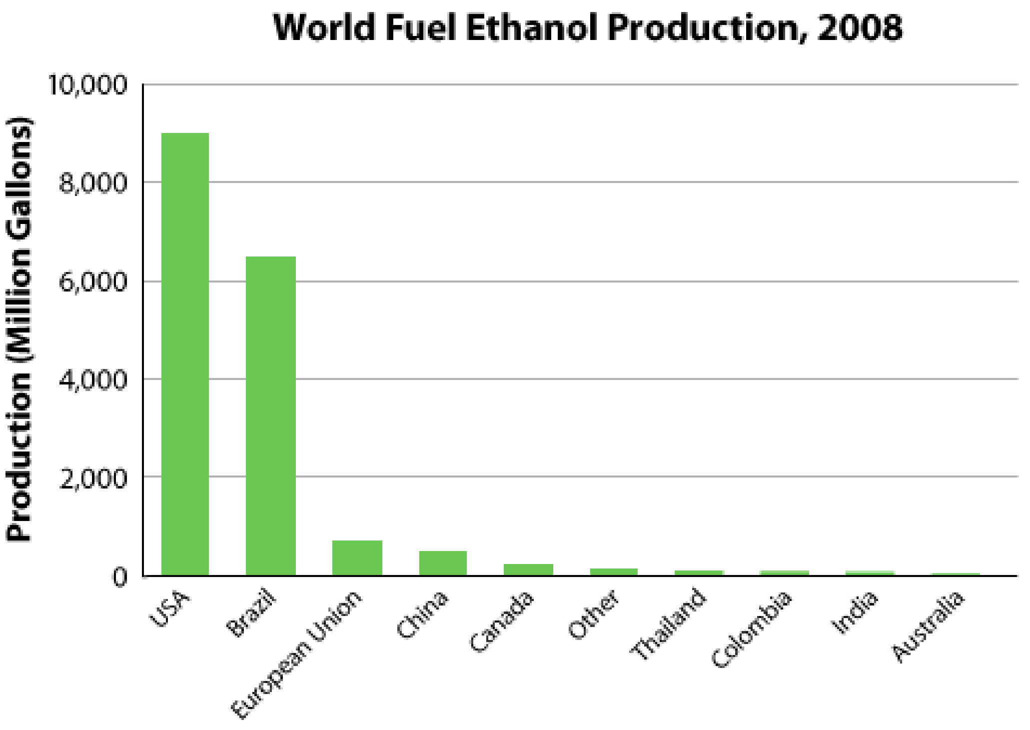
BIO ETHANOL

Production of vehicles using Alchool and different blends

(strong decrease in 1990s)

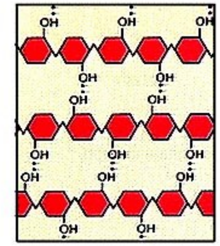
In 2000s Flexible fuel Vehicles

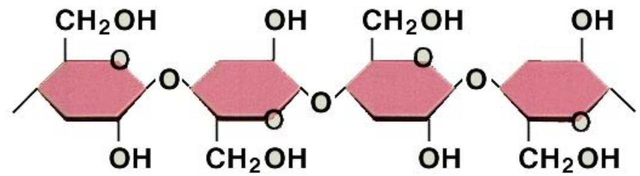
Development of a mature technology for sugar-based fermentation



Bioetanolo

Cellulosa





- bioetanolo
  - Da mais
  - Da canna da zucchero
  - Da biomassa cellulosica

BIO ETHANOL

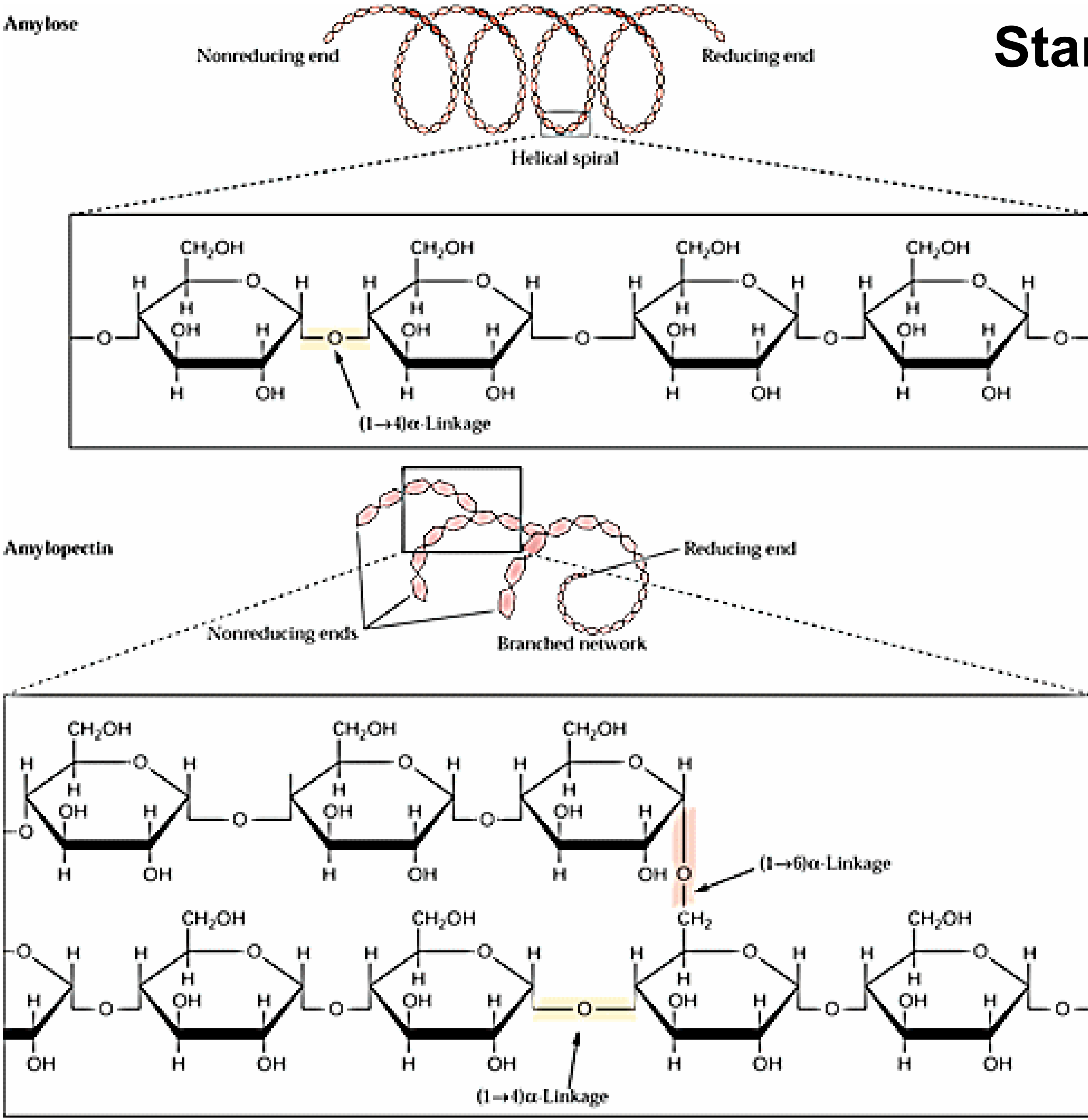
2nd market for Ethanol – from Corn in US

Use of ethanol blends gasoline (E10, E85)

Biological Substrate – Starch glucan polymers

Use of starchy seeds

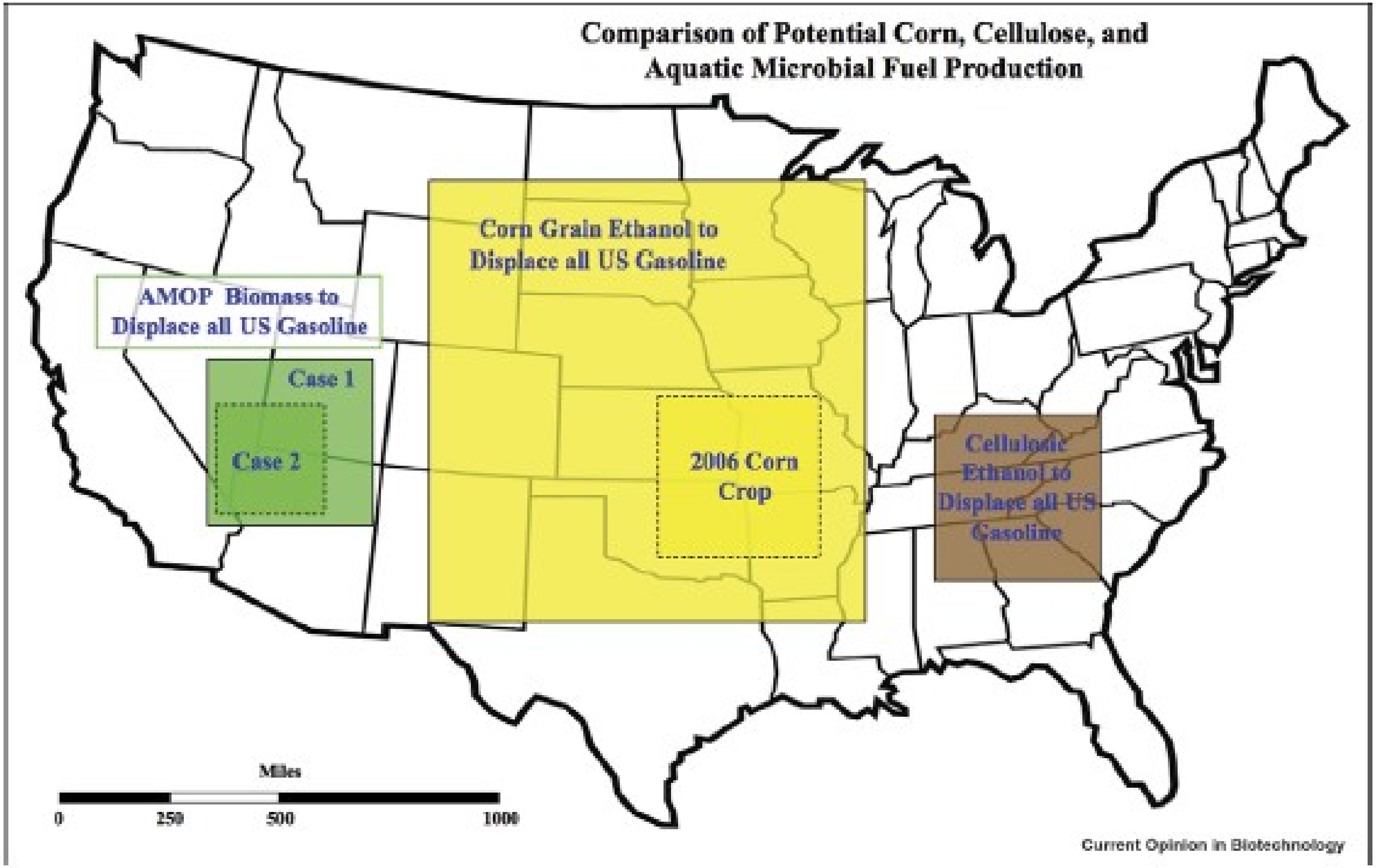
Starch Biosynthesis



Starch is the reserve molecule in the chloroplast (and amyloplasts)

BIO ETHANOL

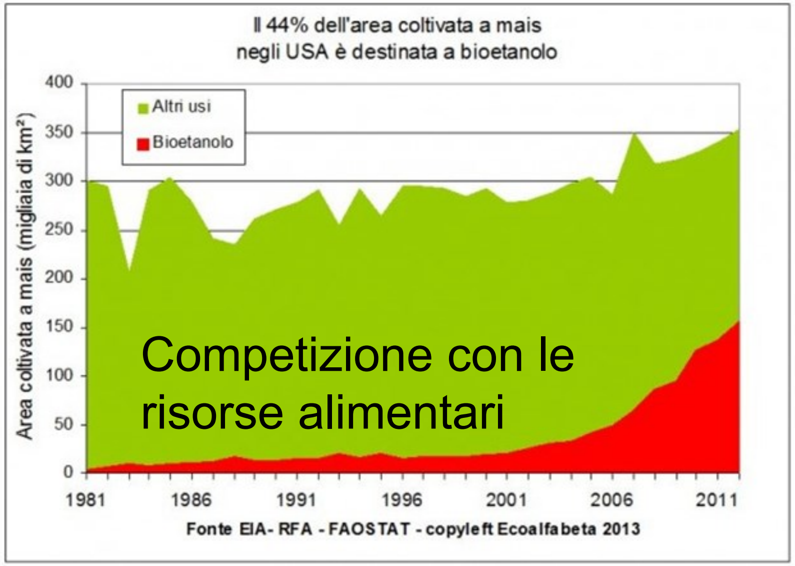
Mature Technology – limited by feedstock availability



Areas needed for cultivation of three biomass sources. Each box represents the area needed to produce a sufficient amount of biomass to convert to liquid fuel to displace all gasoline used in the USA (2006 figures) on an energy basis. Data taken from ref 24.

Problems

1)



Il 44% dell'area coltivata a mais
negli USA è destinata a bioetanolo
Altri usi
Bioetanolo
Area coltivata a mais (migliaia di km²)
400
350
300
250
200
150
100
50
0
1981
1986
1991
1996
2001
2006
2011
Fonte EIA- RFA - FAOSTAT - copyleft Ecoalfabeta 2013
Competizione con le
risorse alimentari

The real advantages of corn-based bioethanol have been questioned

EROI – Energy return of investment

For corn bioethanol it has been estimated to be ≈ 1

This means that at the end we obtain more or less the same amount of energy we invested

Energy invested:

Cultivation,
Fertilization
Harvesting
EtOH production and extraction

BIO ETHANOL

Need of alternative feedstock

Use of whole Biomass not only seeds or sucrose

obtained from agricultural residues, wood, municipal waste, energy crops

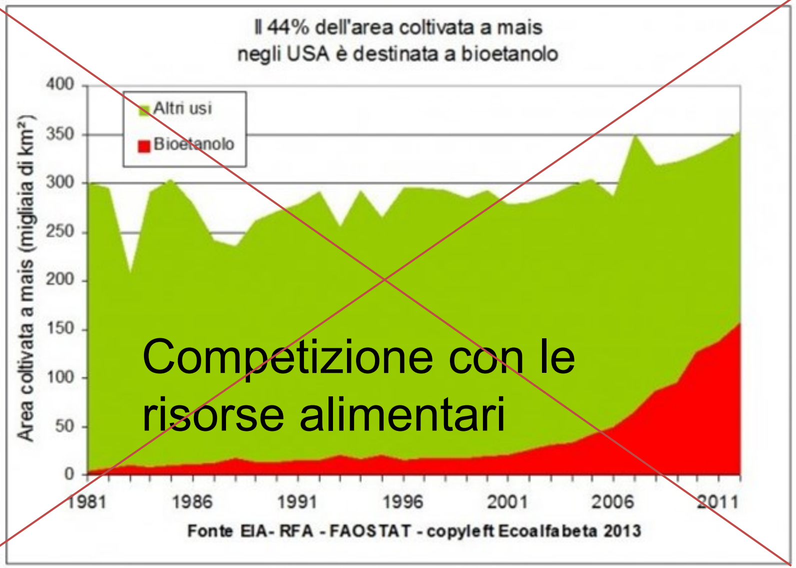
This are composed by

Cellulose	40-50%
Hemicellulose	25-35%
Lignin	15-20%

In this case the feedstock availability would be much higher

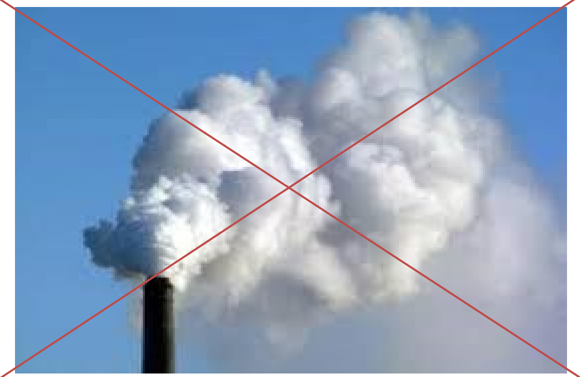
Problems

1)



Competizione con le risorse alimentari

3)



2) La cellulosa è rinnovabile, economica e globalmente disponibile per 50 milioni di tonnellate/ anno.

5)

4) È possibile sfruttare terreni in zone con climi non adatti alla produzione di mais e affini

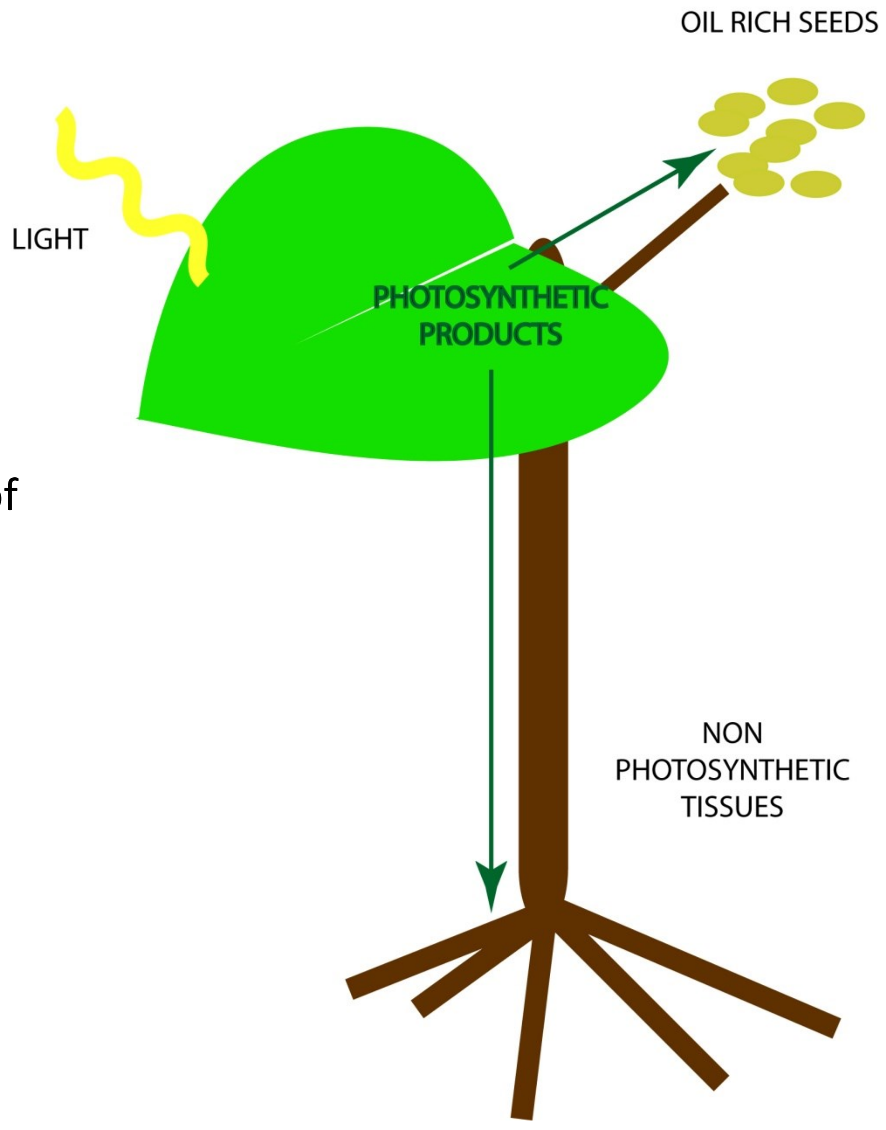
BIO ETHANOL

Lignocellulosic ethanol

use of wood industry / agricultural / municipal solid waste could contribute substantially to fuel consumption

1.3-2.3 billion tons of cellulosic biomass -> 30-50% of US gasoline consumption

All US corn production will cover only 12% gasoline demand



BIO ETHANOL

2nd generation Biofuels, use of Lignocellulose biomasses

BIOENERGY CROPS

– they produce a lot of biomass

- They grow on marginal land, have reduced fertilization demands

Table 1. Comparison of biofuel sources

Biofuel generation	First (crop species)	Second (grasses and trees)	Third (algae)	Refs
Primary products	Bioethanol Biodiesel	Bioethanol Solid fuel Hydrogen gas	Biodiesel Hydrogen gas	
Secondary products	Biomethane Distillers grain Animal feed		Bioethanol Biomethane Glycerol Animal feed Pigments	[57,71]
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Land requirement	High-quality agricultural land	Marginal land	Low-quality land	
Other requirements	Freshwater source	Extensive processing Freshwater source	High sunlight irradiance Close proximity to sea water CO_2 source	

BIO ETHANOL

Lignocellulose biomasses

-Cellulose – structural polymer in plants
Highly insoluble, organized in crystalline fibers

Mixed with emicellulose

Often protected by lignins in woody tissues

Table 1. Comparison of biofuel sources

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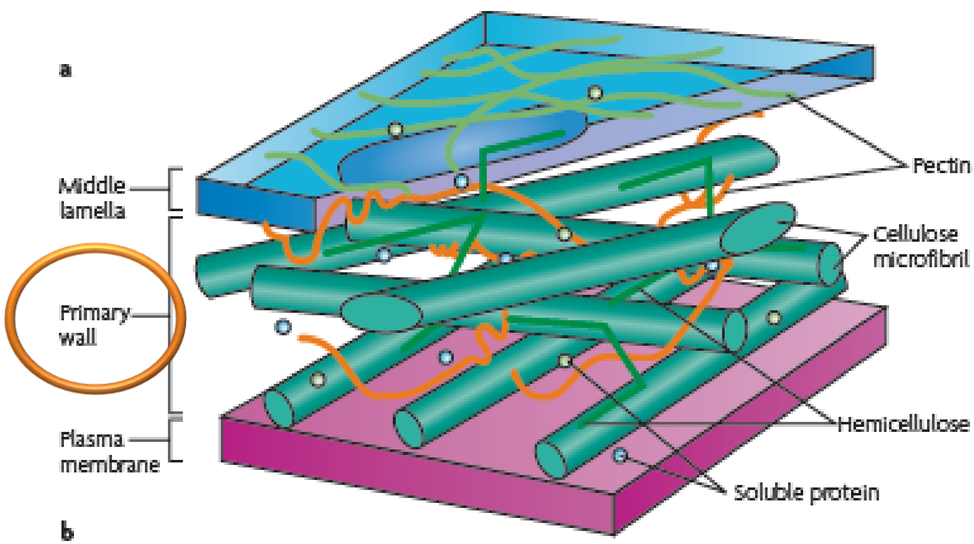
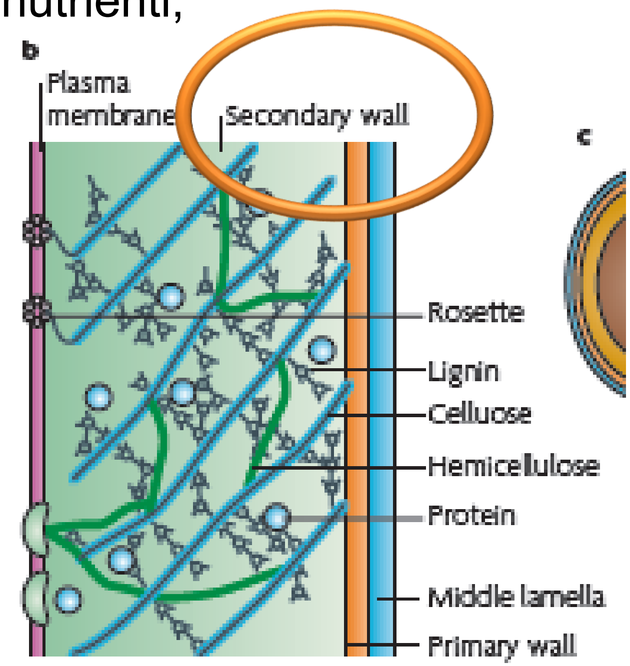
La parete cellulare



b

È fondamentale per:

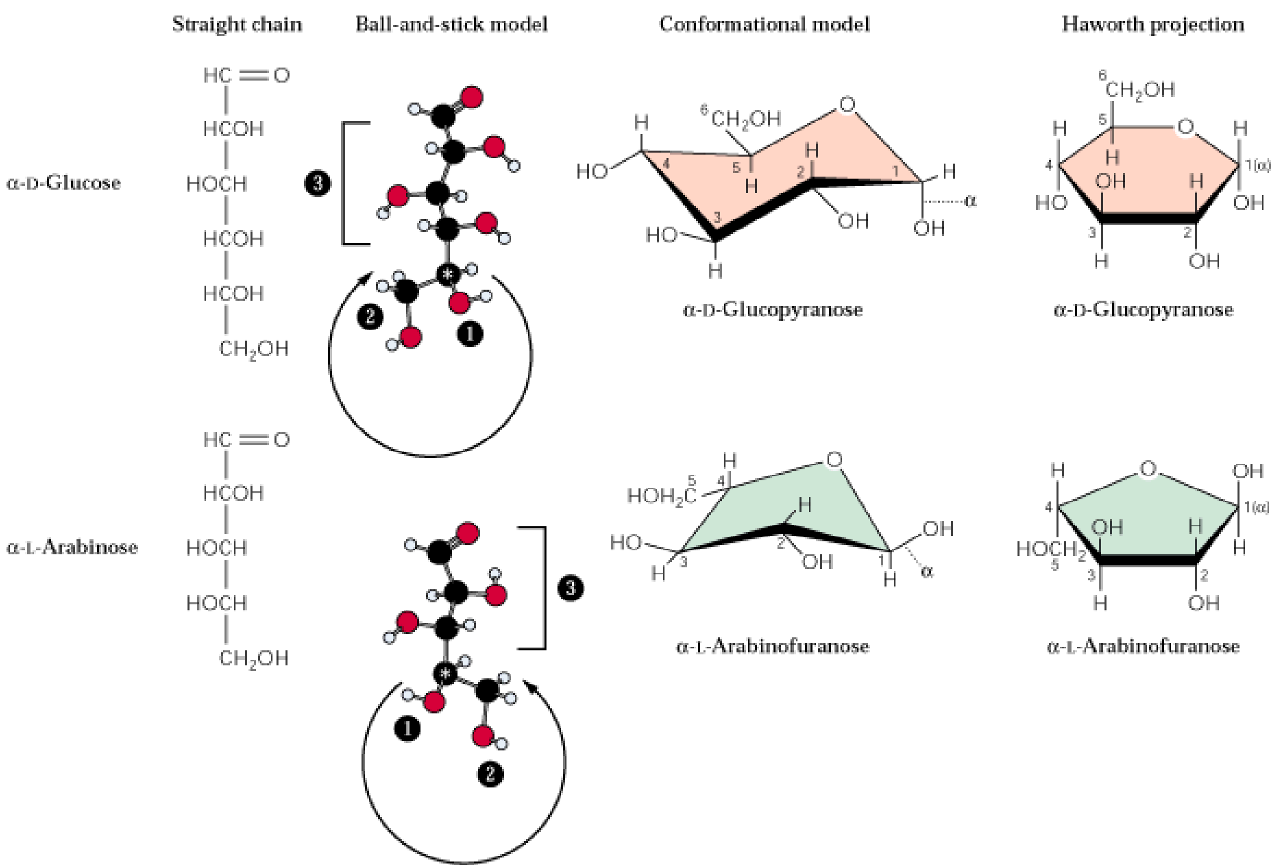
- l'integrità strutturale della pianta,
- la difesa dai patogeni,
- il trasporto di acqua e nutrienti;



Ci sono almeno 2 layers :

1) PRIMARIA = cellulosa, emicellulosa e pectina
2) SECONDARIA= cellulosa, emicellulosa e lignina

Cell wall is composed by carbohydrates



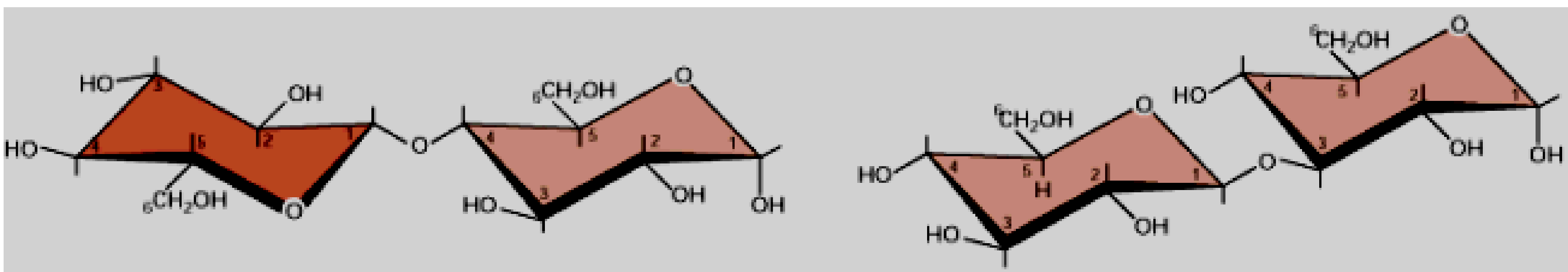
C 1 is the anomeric Carbon (bound to 2 oxygen atoms).

α, β: depends on the position of –OH group bound to anomeric carbon.

Sugars polymerization change the monomer characteristics

A di-saccharide is defined by the : a) the type of bond; anomeric configuration

Ex. Cellobiose is a β-D-glucosyl (1→4)-D-Glucose.
An anomeric bind is formed when the C1 bind to a OH bound to C4 in another D glucose.
Only one glucose unit is blocked in β configuration; the other one is free to rotate and is not named.



Cellobiose
(β-D-Glucosyl-(1→4)-D-glucose)

Laminaribiose
(β-D-Glucosyl-(1→3)-D-glucose)

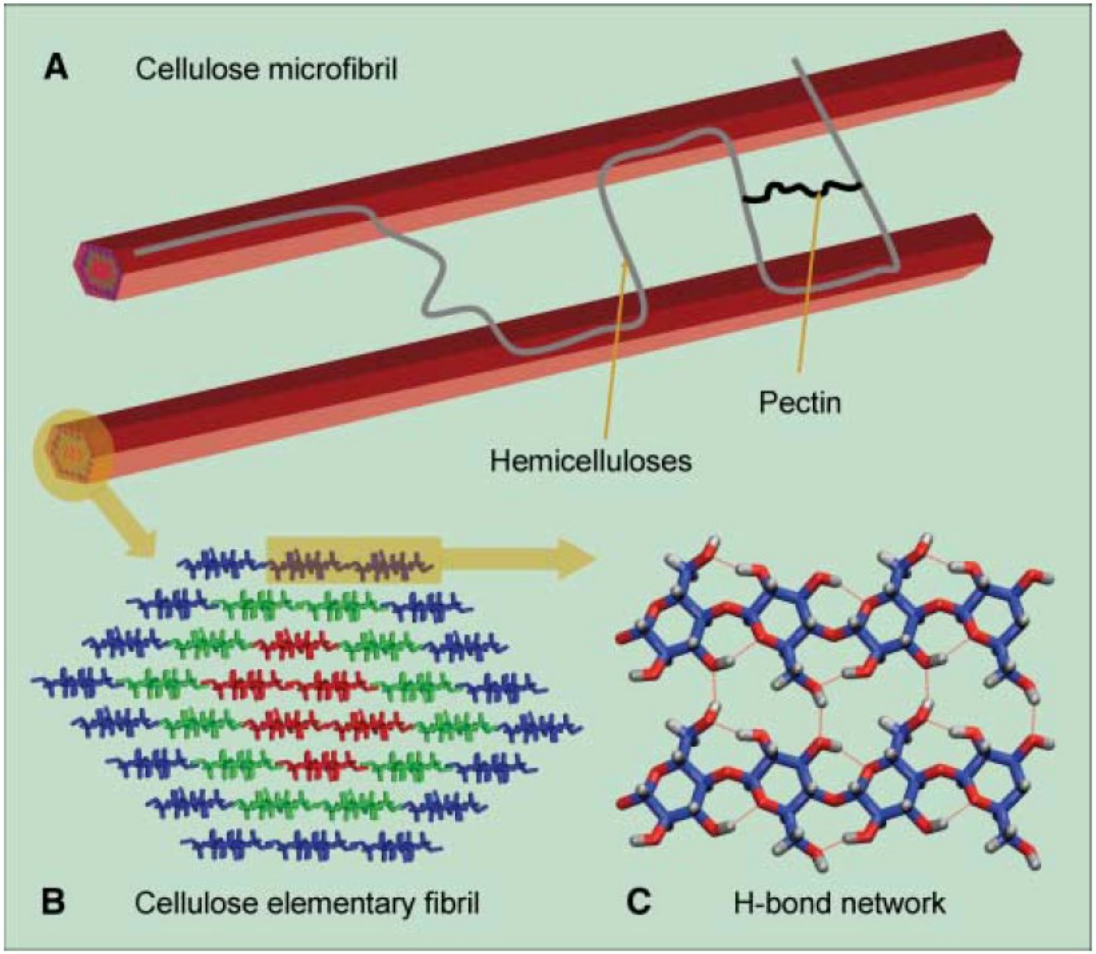
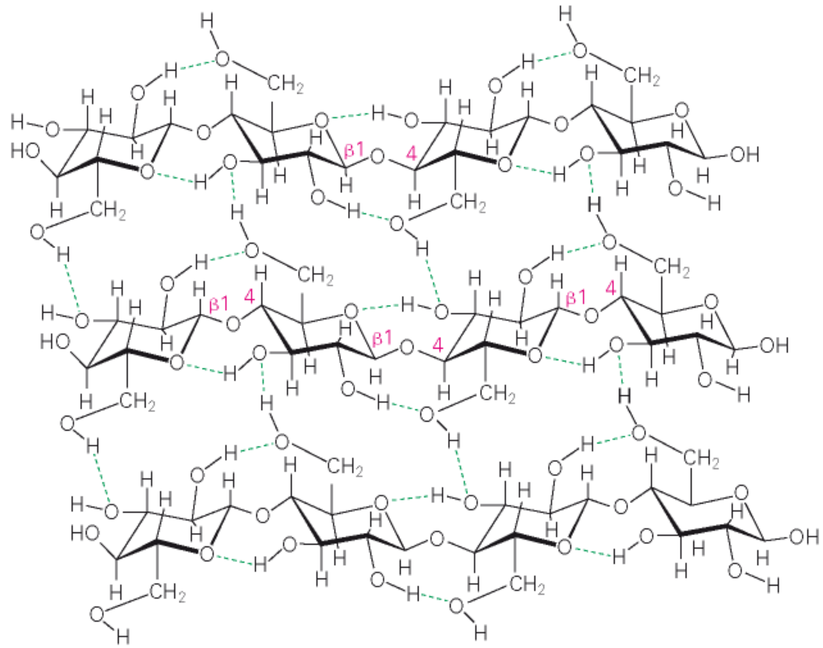
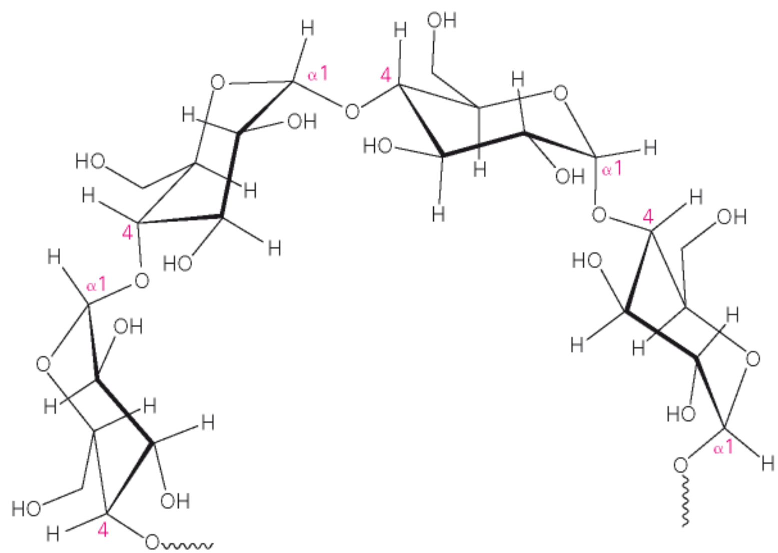


Fig. 2. (**A**) A simplified model showing the interaction of the major polysaccharides in the cell wall. (Lignin is not shown here because its interactions are not well established.) In this system, hemicelluloses are closely associated to the surface of the rigid cellulose crystallite forming the microfibril network. Pectins are cross-linked polysaccharides forming a hydrated gel that "glues" the cell-wall



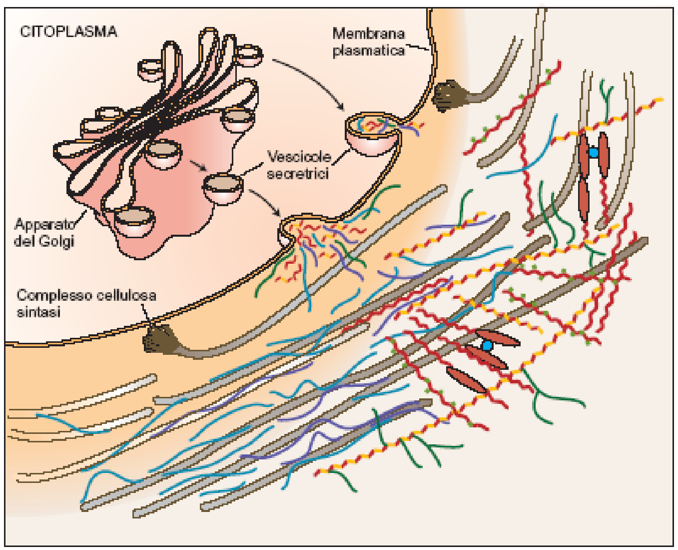
Cellulosa
(legami β-1,4)



Amido e glicogeno
(legami α-1,4)

This wide network of non-covalent bonds between glucans within a micro-fibril gives remarkable properties to cellulose. Cellulose has a very large resistance to torsion, similar to steel. It is relatively insoluble, stable and resistant to chemical and enzymatic attacks

Excellent material to build a strong cell wall resistant to mechanical stress and pathogens



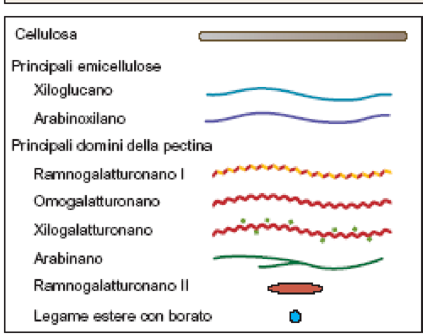
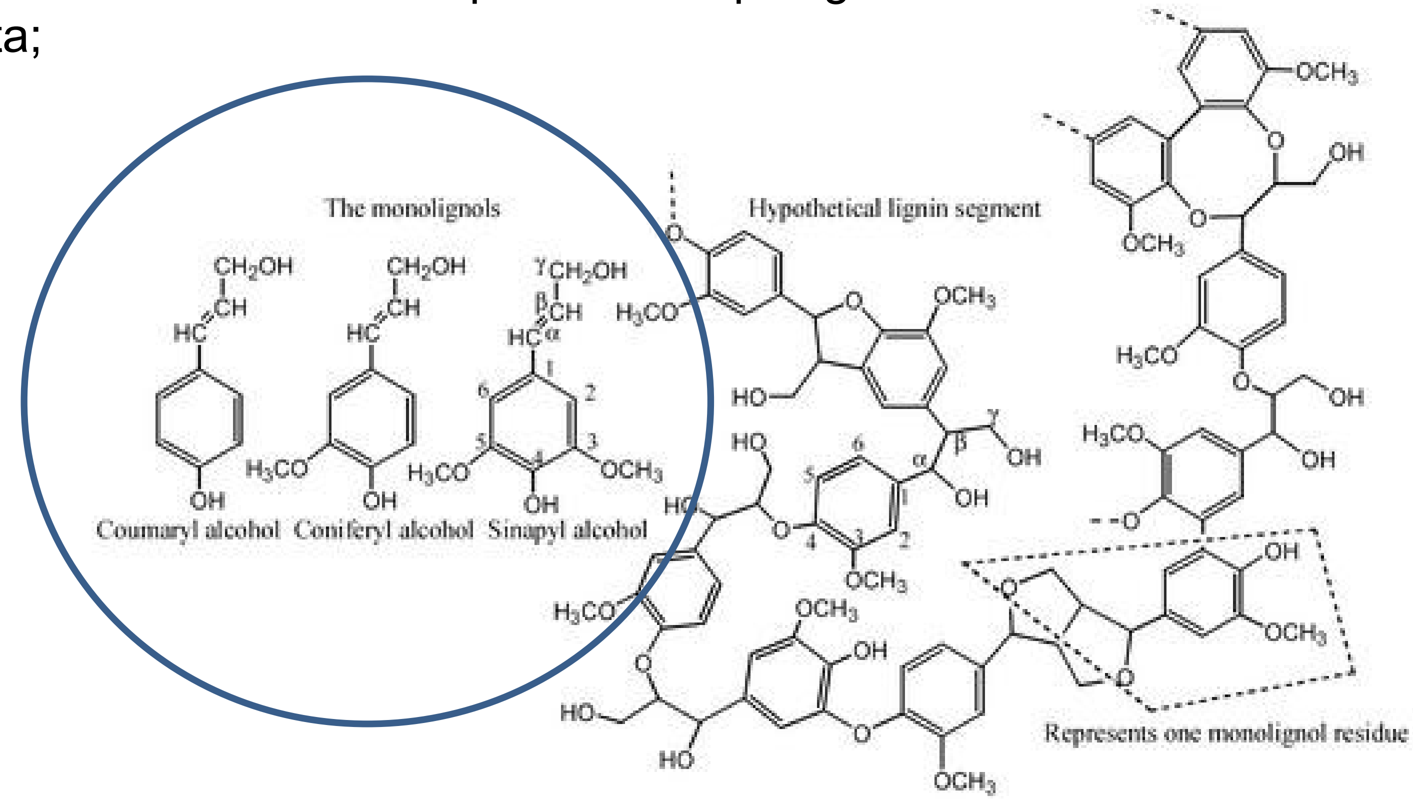


Figura 15.4 Diagramma schematico delle componenti principali della parete cellulare primaria e della loro possibile dislocazione. Le microfibrille di cellulosa (barre grigie) sono sintetizzate sulla superficie della cellula e sono rivestite da emicellulose (fasci blu e porpora) che legano le microfibrille. Le pectine (fasci gialli, rossi e verdi) formano una matrice di ancoraggio intermedia che controlla la distanza fra le microfibrille e la porosità della parete. Le pectine e le emicellulose sono sintetizzate nell'apparato del Golgi e riversate nella parete tramite vescicole che si fondono con la membrana plasmatica e che quindi depositano questi polimeri sulla superficie della cellula. Per maggior chiarezza viene mostrato a sinistra un dettaglio dell'intreccio di emicellulose e cellulose e della componente pectica. (Da Cosgrove 2005).

Lignina

- È un polimero fenolico non lineare, composto da una serie di **subunità** distinte le cui abbondanza relativa persino tra tipologie cellulari diverse nella stessa pianta;

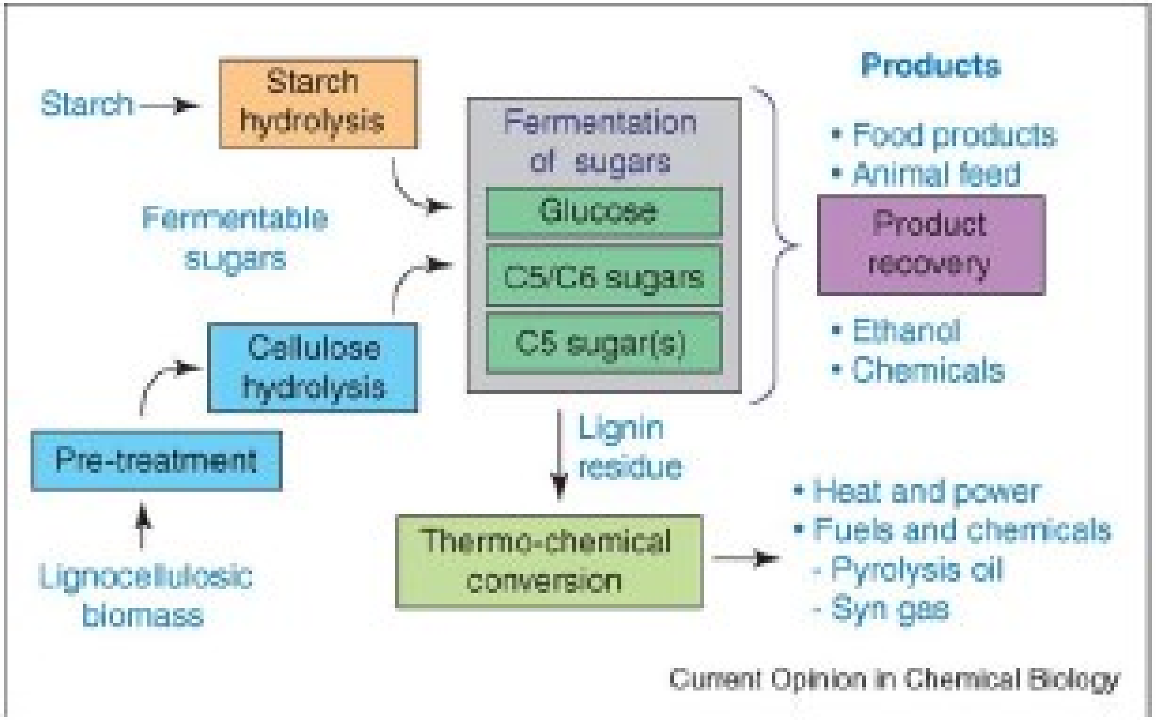


BIO ETHANOL

Industrial process:

3 major steps:

1. Thermochemical pretreatment to increase accessibility of cellulose
2. Saccharification (enzymatic)
3. Fermentation of released sugars to ethanol



Schematic of biomass and starch processing that could occur in a biorefinery.

Problemi

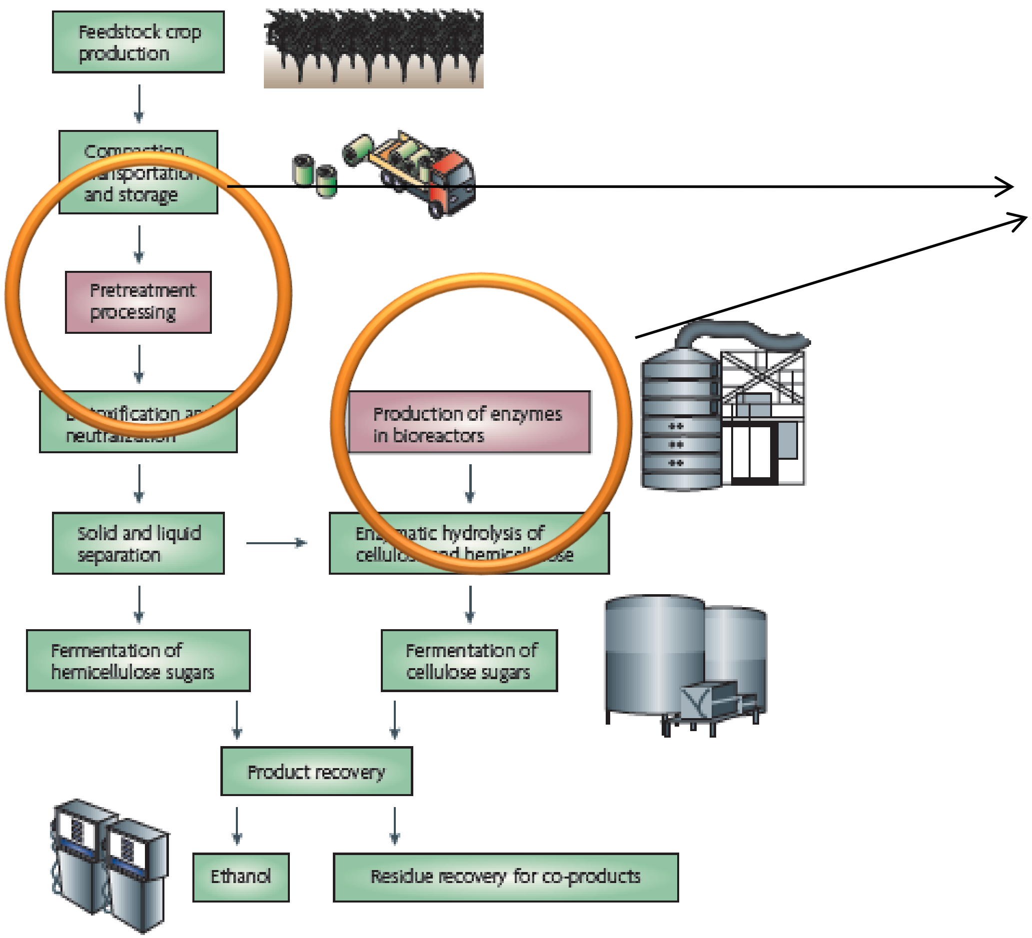
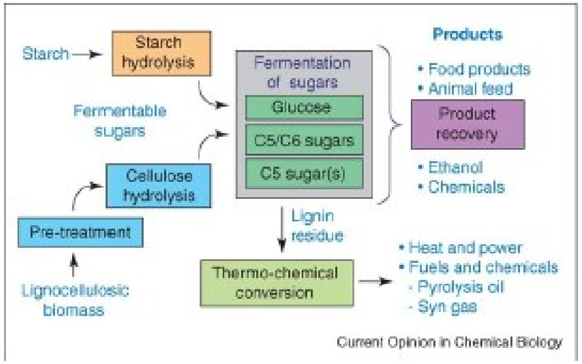


Figure 2 | **Overview of cellulosic ethanol production.** Flow chart showing the steps in the production of cellulosic ethanol from feedstock crops.

COSTI ELEVATI E SCARSA RESA per la presenza della PARETE CELLULARE nelle cellule vegetali

BIO ETHANOL

1. Thermochemical pretreatment to increase accessibility of cellulose



Schematic of biomass and starch processing that could occur in a biorefinery.

Raw biomass is completely recalcitrant to enzymatic degradation

Disrupt cell wall and improves enzymatic access

There is a correlation between removal of lignin and hemicellulose and the digestibility of cellulose

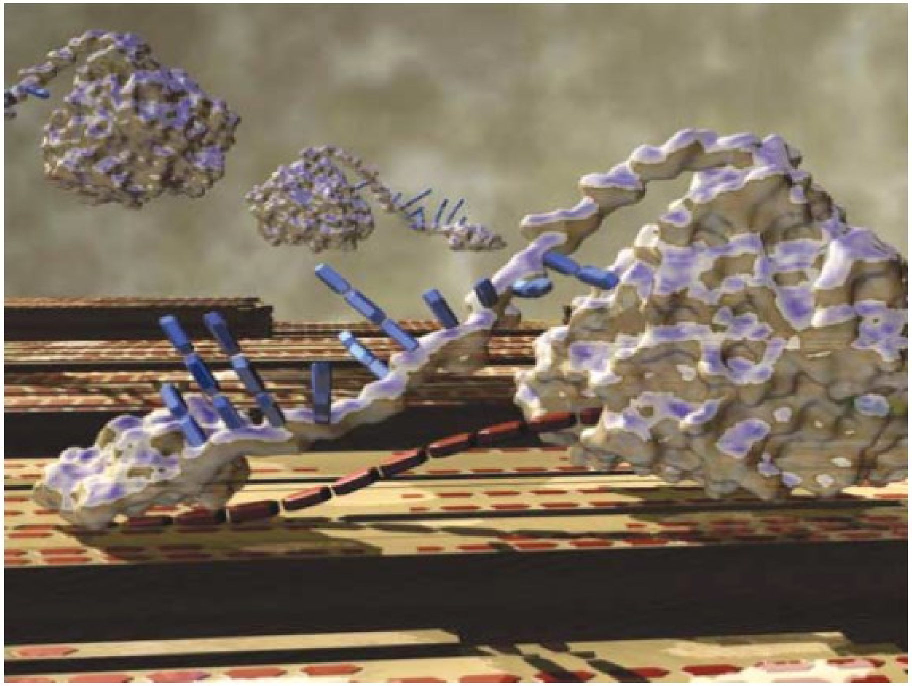
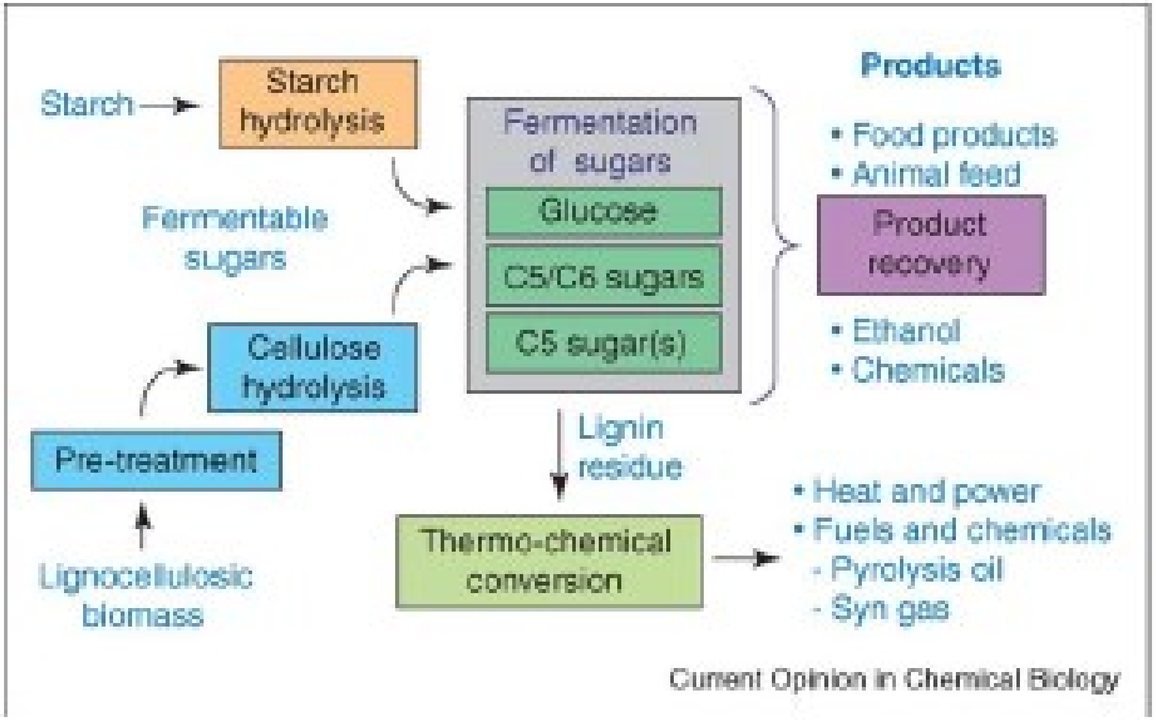


Fig. 3. Artistic concept of an exoglucanase (the *T. reesei* cellobiohyrolase I) acting on crystalline cellulose. In this depiction, the carbohydrate-binding module (left) recognizes and binds to the cellulose surface. By a process not fully understood, a single chain of cellulose is "decrystallized" and directed into the active-site tunnel of the catalytic domain (right). This enzyme is thought to proceed along a cellulose chain cleaving one cellobiose unit per catalytic event until the chain ends or the enzyme becomes inactivated (40, 41).

BIO ETHANOL

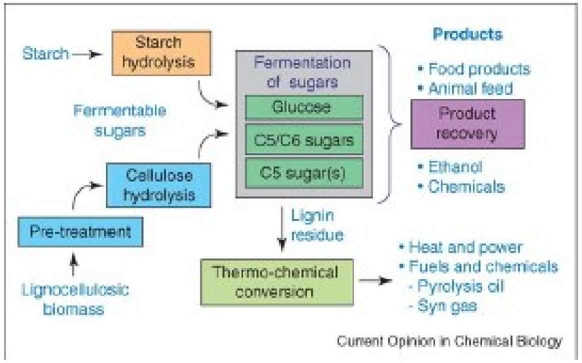


Schematic of biomass and starch processing that could occur in a biorefinery.

Lignin residue is not usable for fermentation

BIO ETHANOL

2. Saccharification (enzymatic)



Schematic of biomass and starch processing that could occur in a biorefinery.

Cellulosases

Endoglucanases – cleave internal β 1-4 bonds
Exoglucanases – act on the reducing and non-reducing ends
β-glucosidases - hydrolyze soluble oligosaccharides (e.g. cellobiose)

In anaerobic bacteria and some fungi these enzymes are complexed forming CELLULOSOMES

BETA RENEWABLES

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World's first commercial-scale cellulosic ethanol plant uses the proesa® technology process

Beta Renewables Crescentino plant validates the PROESA® process at commercial scale:

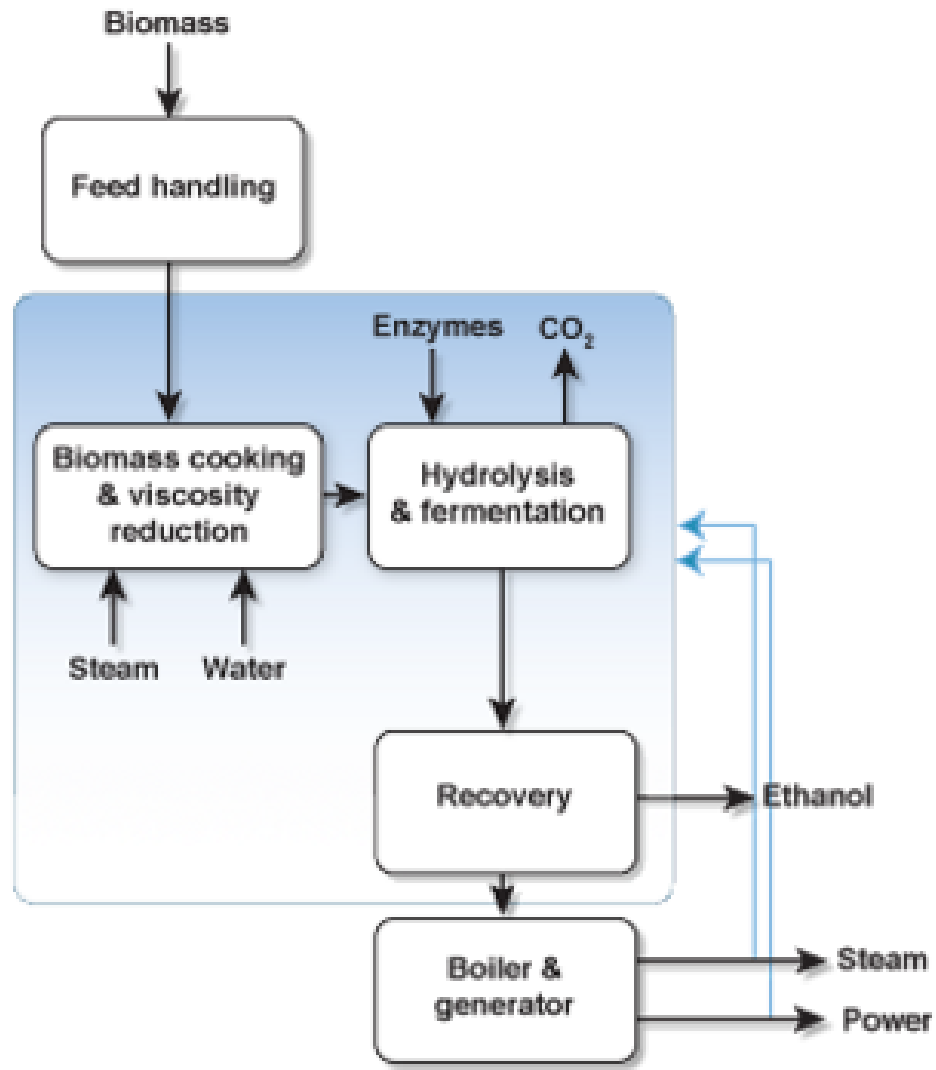
- Started operations, Q4 2012
- 60,000 metric tons per year (20 million gallons); initially 40,000 tons
- Non-food biomass (Arundo donax and wheat straw)
- Industry-leading economics

The world's first commercial-scale cellulosic ethanol plant, in Crescentino Italy, started operations in Q4, 2012. Our PROESA® process allows it to deliver superior economics in converting non-food biomass to sugars for the production of bio-ethanol or bio-chemicals.

© 2012 Beta Renewables S.p.A.

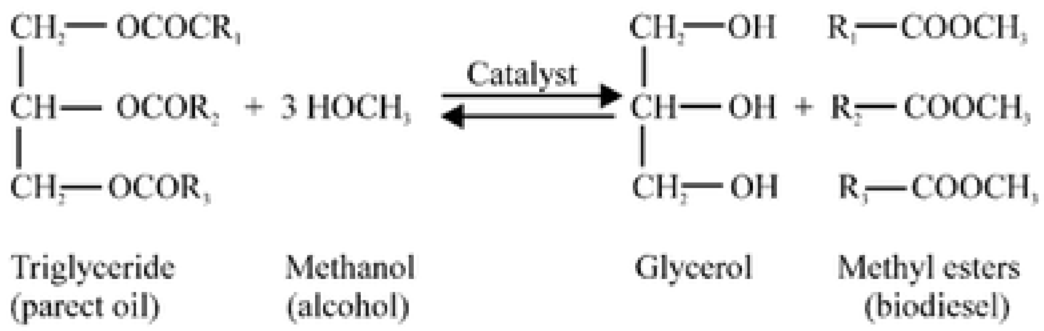
http://www.betarenewables.com/PROESA-technology.html

PROESA® combines an enzymatic pre-treatment process with fermentation. Our process runs significantly faster than other enzymatic hydrolysis approaches, is acid-and alkali-free and has minimal byproducts. Our parameters are adjustable, providing flexibility in the desired output of C5 sugars, C6s and lignin to be used in the production of ethanol or chemicals. Our approach provides better overall performance and economics than other hydrolysis or gasification technologies that we are aware of. Our PROESA® technology and process is covered by 21 pending patents.



3. Biodiesel

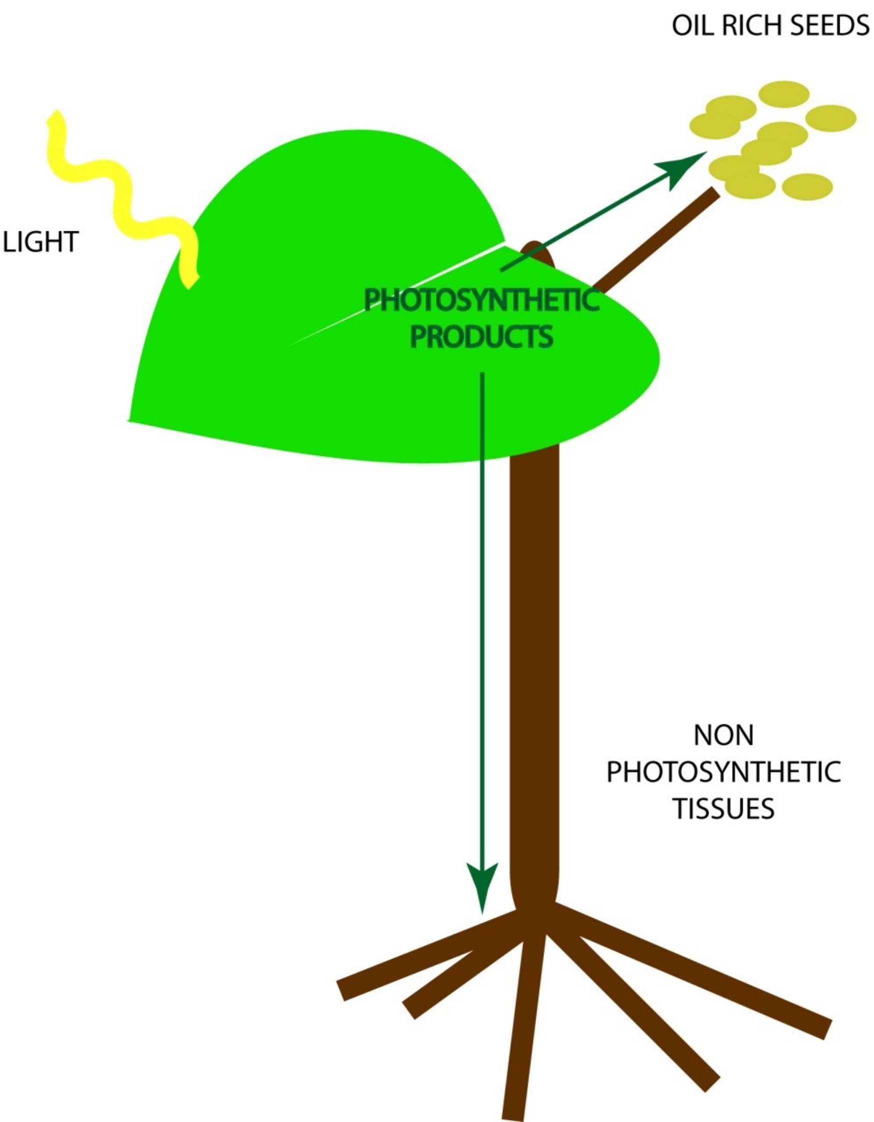
Biodiesel is the product of Triacylglycerol (TAG) transesterification



Biodiesel can be produced from triacylglycerols of any origin

Major issue is feedstock availability

Biodiesel



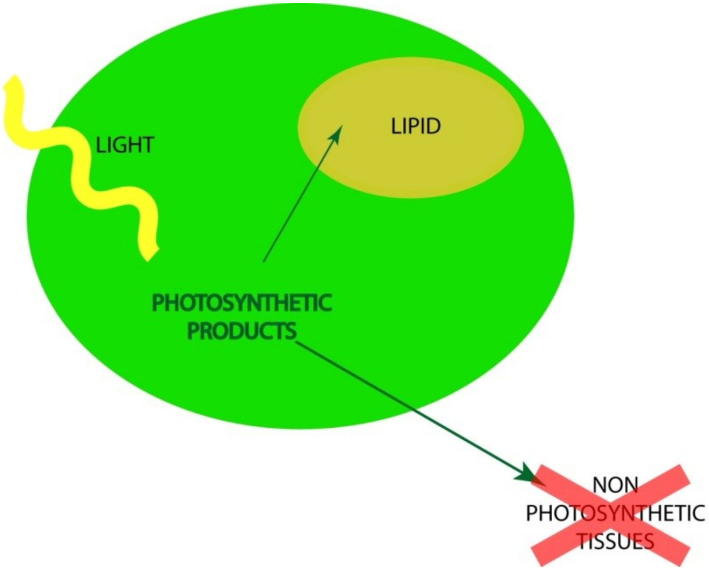
Most Biodiesel is currently produced from crop plants, using oil rich seeds

Non all tissues are photosynthetically active

Only a fraction of the energy goes to energy storage (Oil rich seeds)

≈ 5% of total biomass

PLANTS vs. MICROALGAE

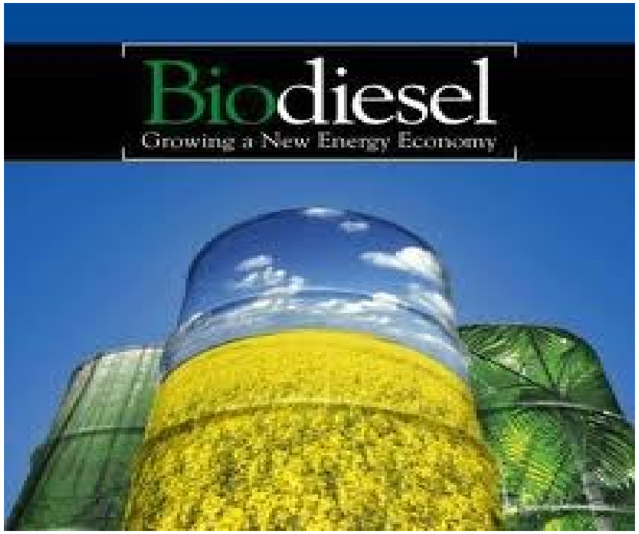


Microalgae are unicellular
All cells are photosynthetically active

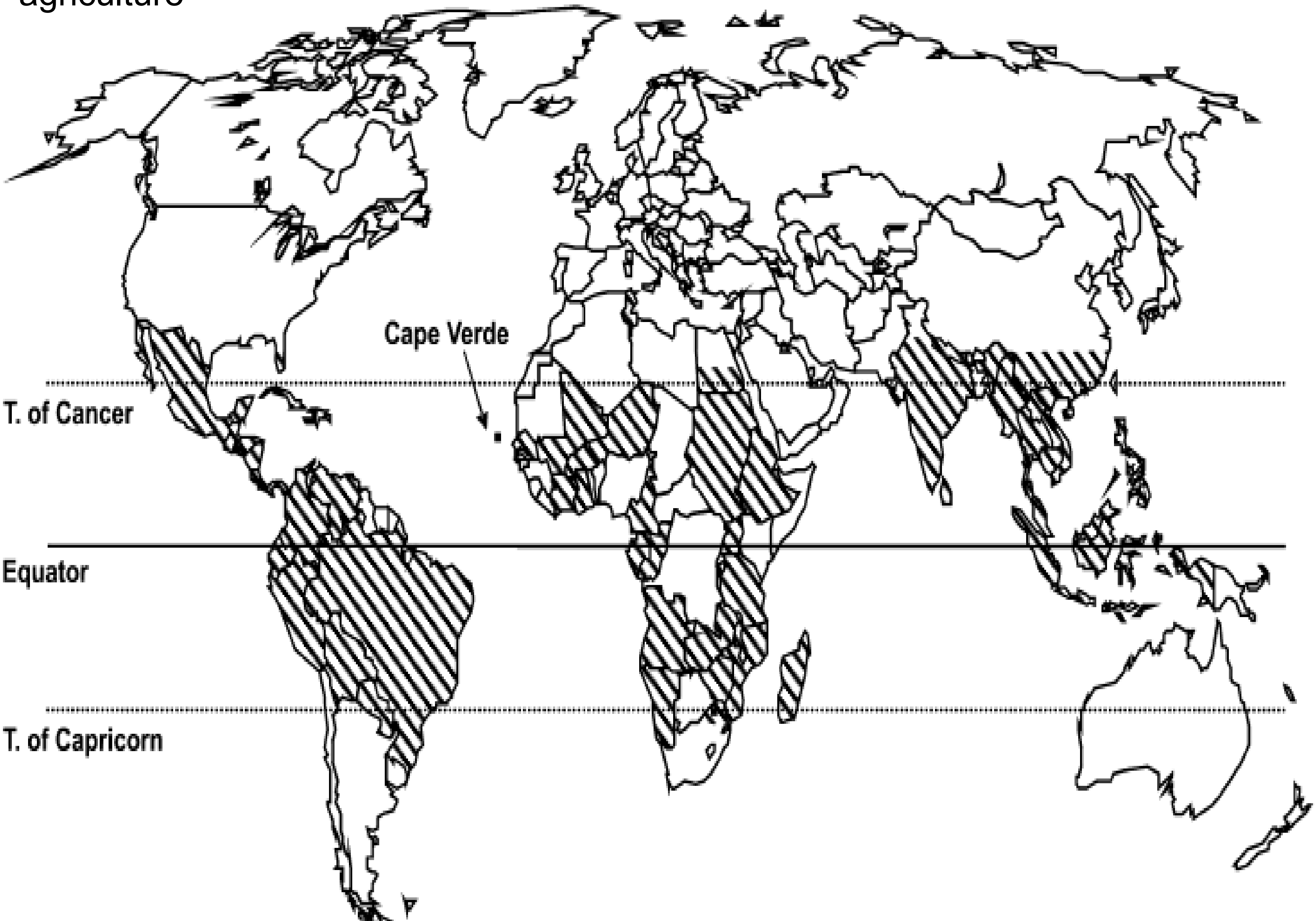
Figure Lipid bodies imaging in *Nannochloropsis gaditana* cells. Red fluorescence corresponds to the chloroplast while the yellow one originates from lipid bodies stained with Nile Red.

Lipids content can reach ≈ 40-80 % of total dry weight

Jatropha curcas as a source of renewable oil



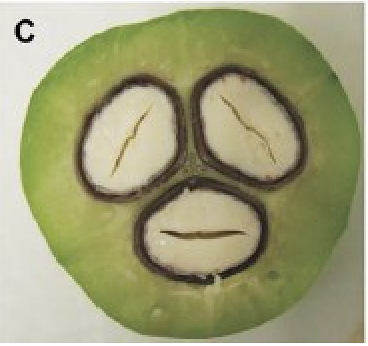
Plant, growing in tropical climates, does not need fertile land nor intensive agriculture



Toxicity of J. curcas seed and the potential value of seed meal

- ***J.curcas* seeds**

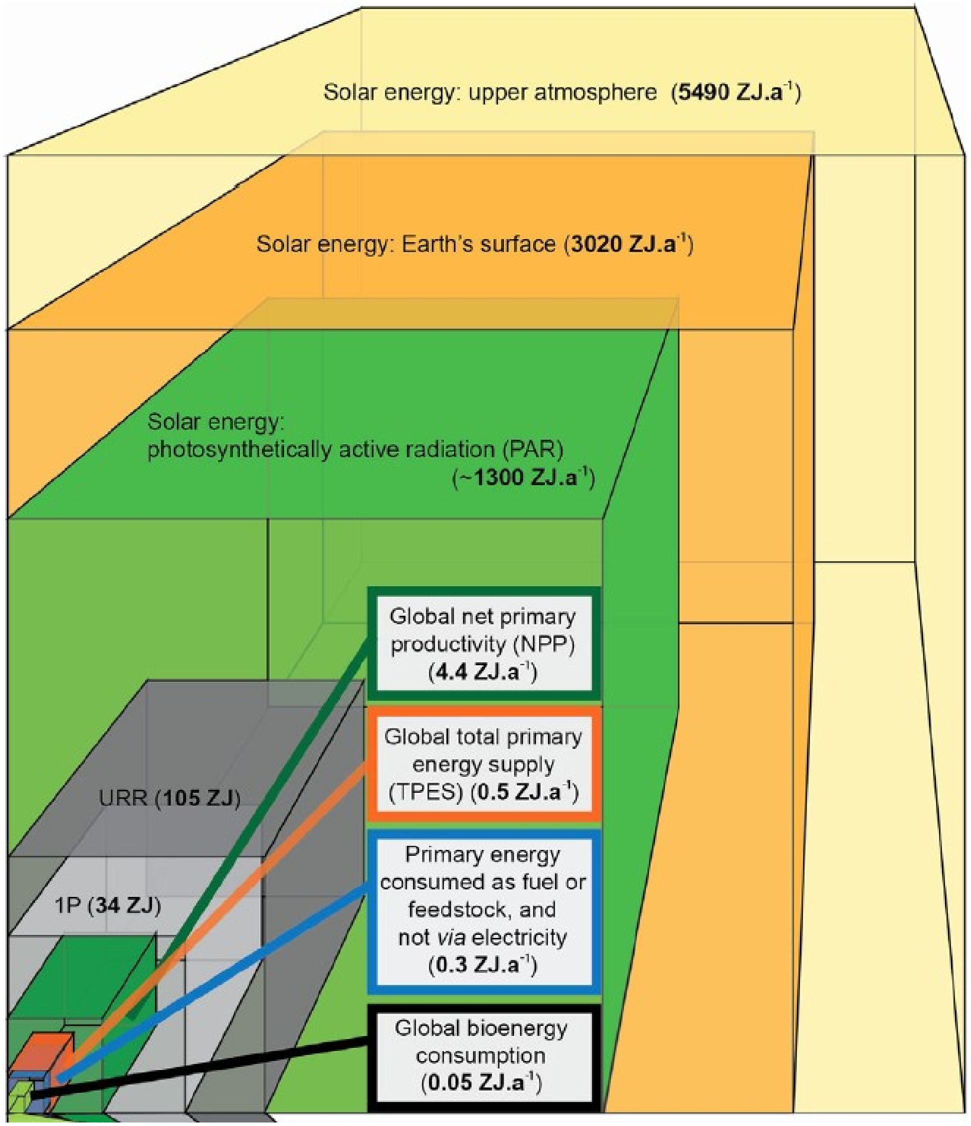
Are protein rich they could be a source of proteins



...but

They are not edible!!
SEED-MEAL obtained after oil extraction is TOXIC and cannot be used as FODDER

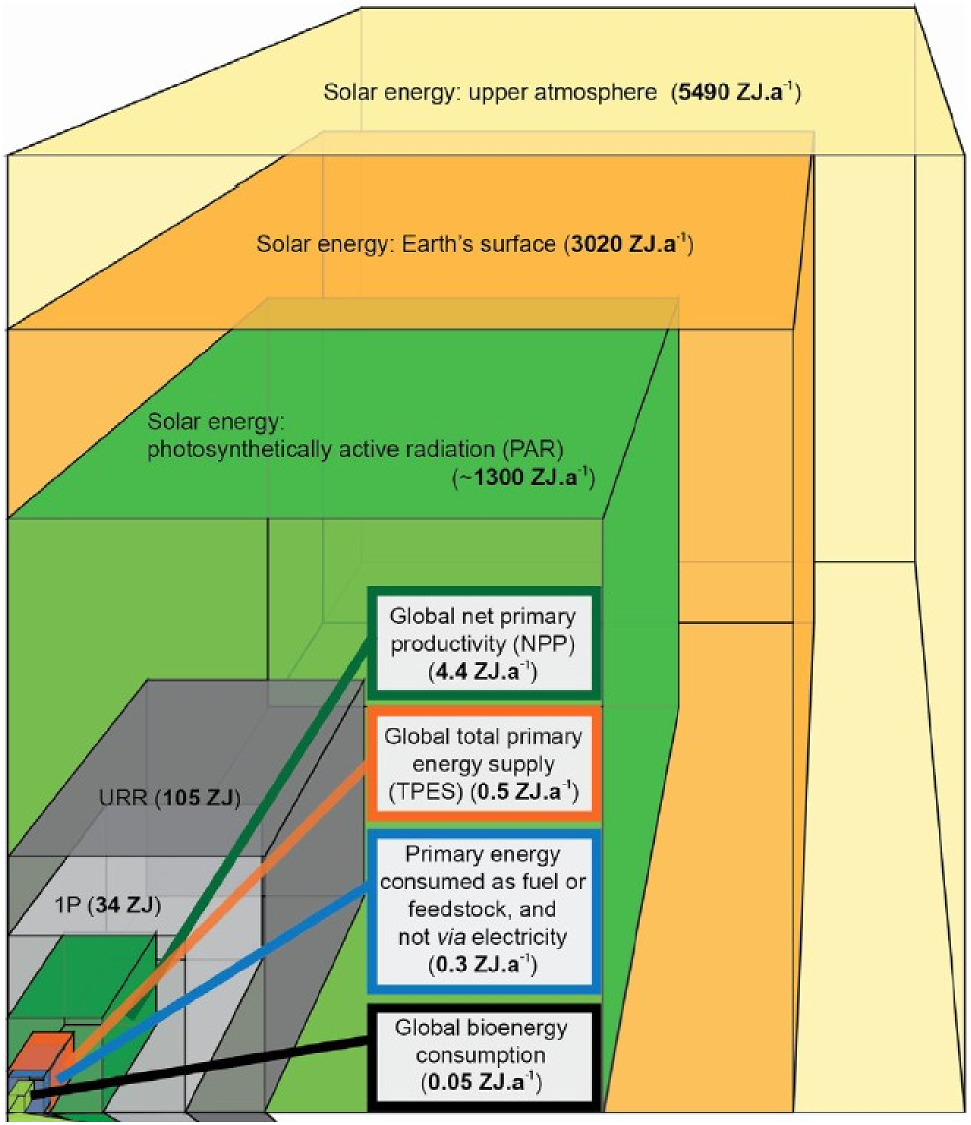
4. Using algae as alternative biomass



Terrestrial NPP = 53-59 GtC / year

1 Kg biomass = 0.45 Kg C = 17.5 MJ

2.1 ZJ



water NPP ≈ 55 GtC / year

1 Kg biomass = 0.47 Kg C = 20 MJ

2.3 ZJ

total NPP ≈ 4.4 ZJ / year

9 times the 0.503 ZJ used every year by the global economy

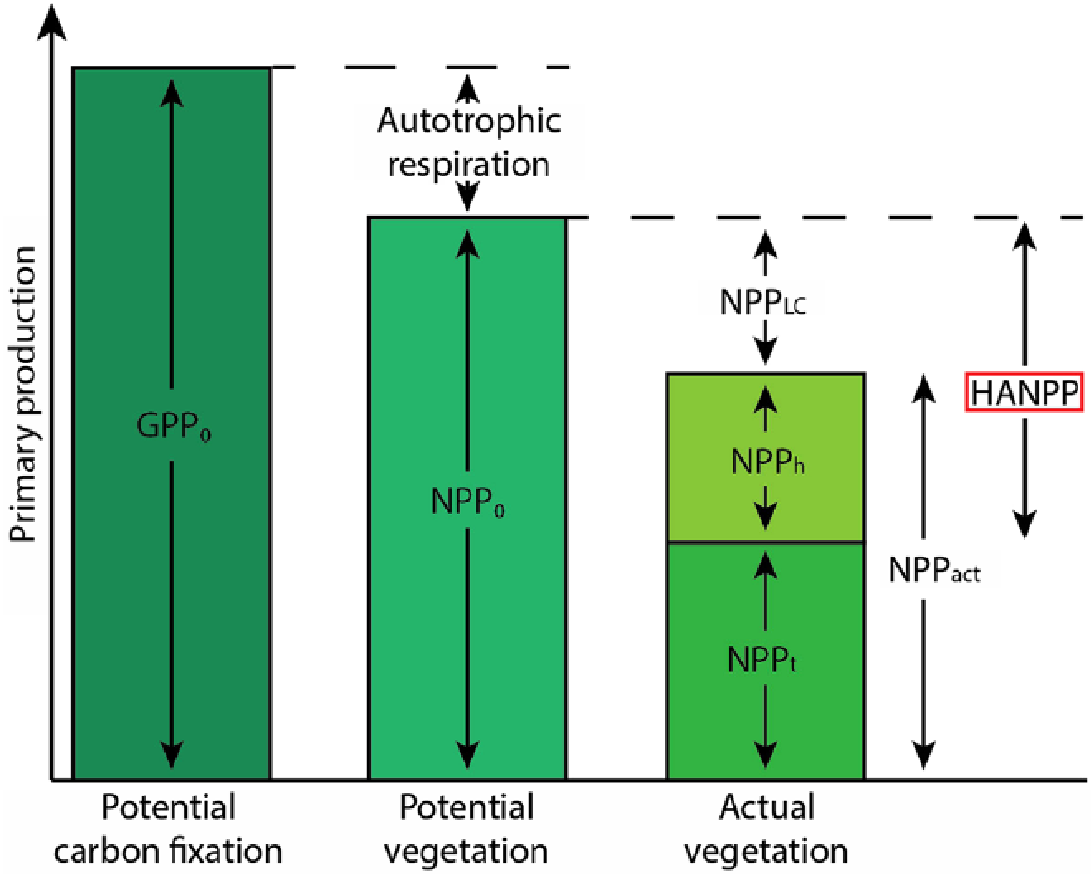


Fig. 3. The standard definition of human appropriation of net primary production (HANPP), graphically represented. Terms are defined in Section 2.6.1. Figure adapted from [42].

Loss in NPP because of human activity (land use changes)

Extraction / destruction of NPP for human purposes (biomass harvest / grazing)

HANPP = human appropriation of net primary production

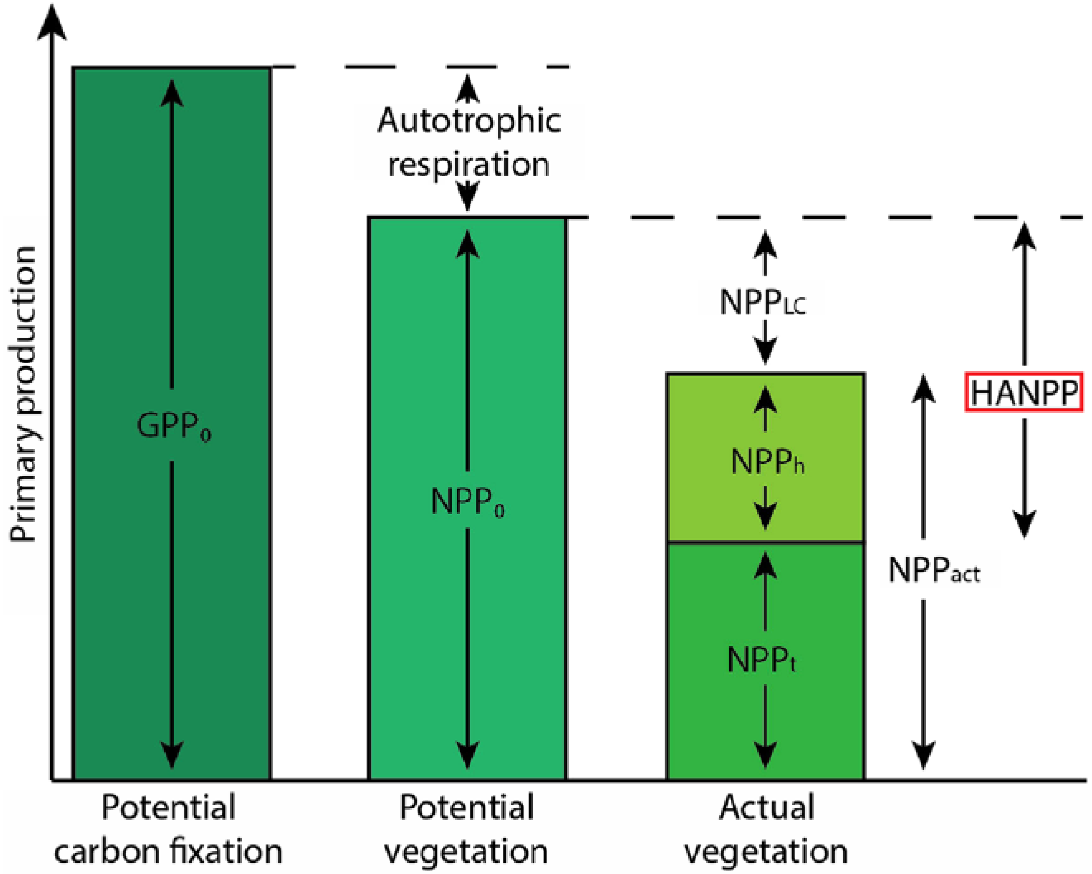


Fig. 3. The standard definition of human appropriation of net primary production (HANPP), graphically represented. Terms are defined in Section 2.6.1. Figure adapted from [42].

Terrestrial HANPP estimated to be 28% of NPP

0.32 KJ energy (64% of global energy demand)

aquatic HANPP estimated to be 8% of NPP (to support fish harvest)

HANPP = human appropriation of net primary production

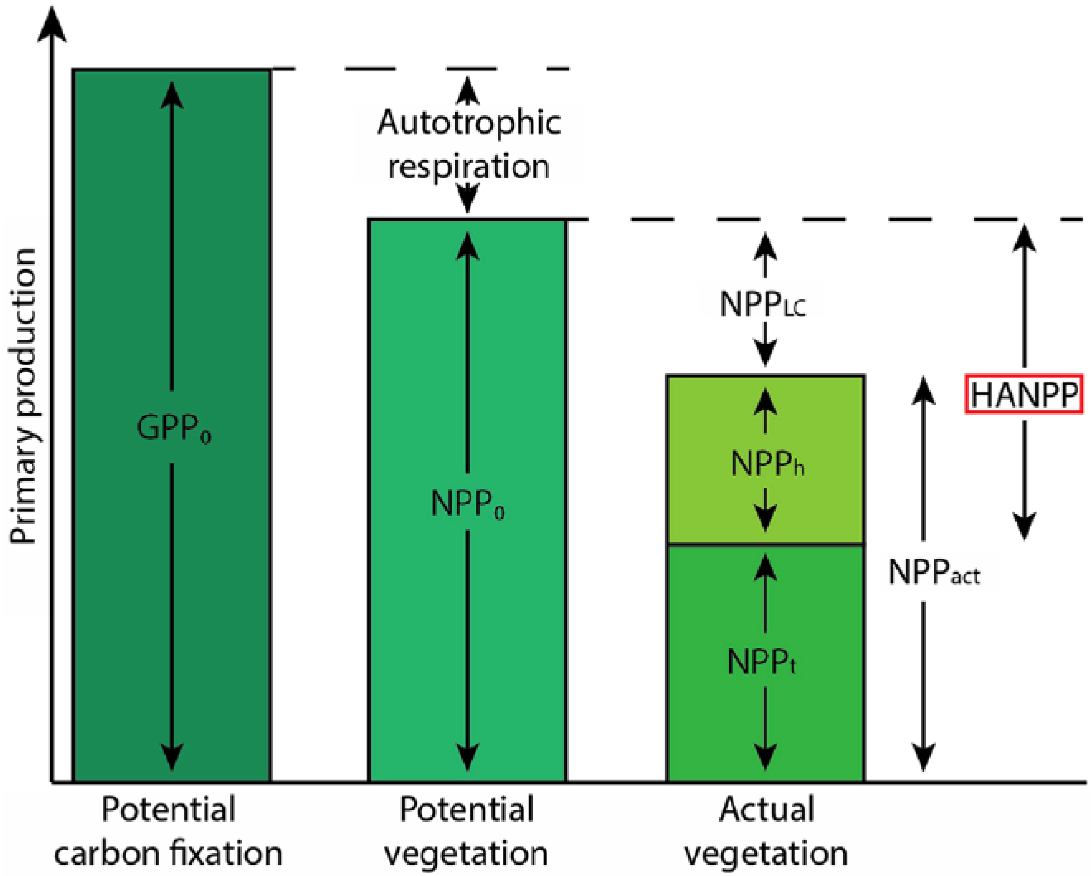


Fig. 3. The standard definition of human appropriation of net primary production (HANPP), graphically represented. Terms are defined in Section 2.6.1. Figure adapted from [42].

High HANPP are a threat for biodiversity and sustainability

e.g. other species need NPP

A sustainable world should use 60% less of HANPP

Increasing HANPP is not the answer for improving our energy demand

Food demand will increase in next decades. There will be no likely no increase in NPP available for energy purposes.

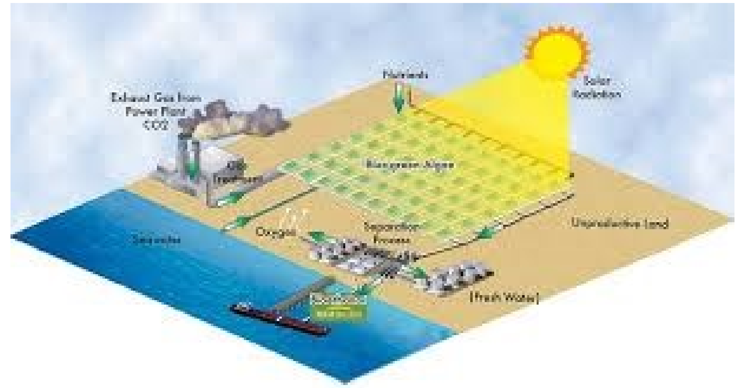
Need for

- Improved efficiency in biomass use
- Increase earth photosynthetic productivity, (e.g. use unproductive areas)

Why algae?

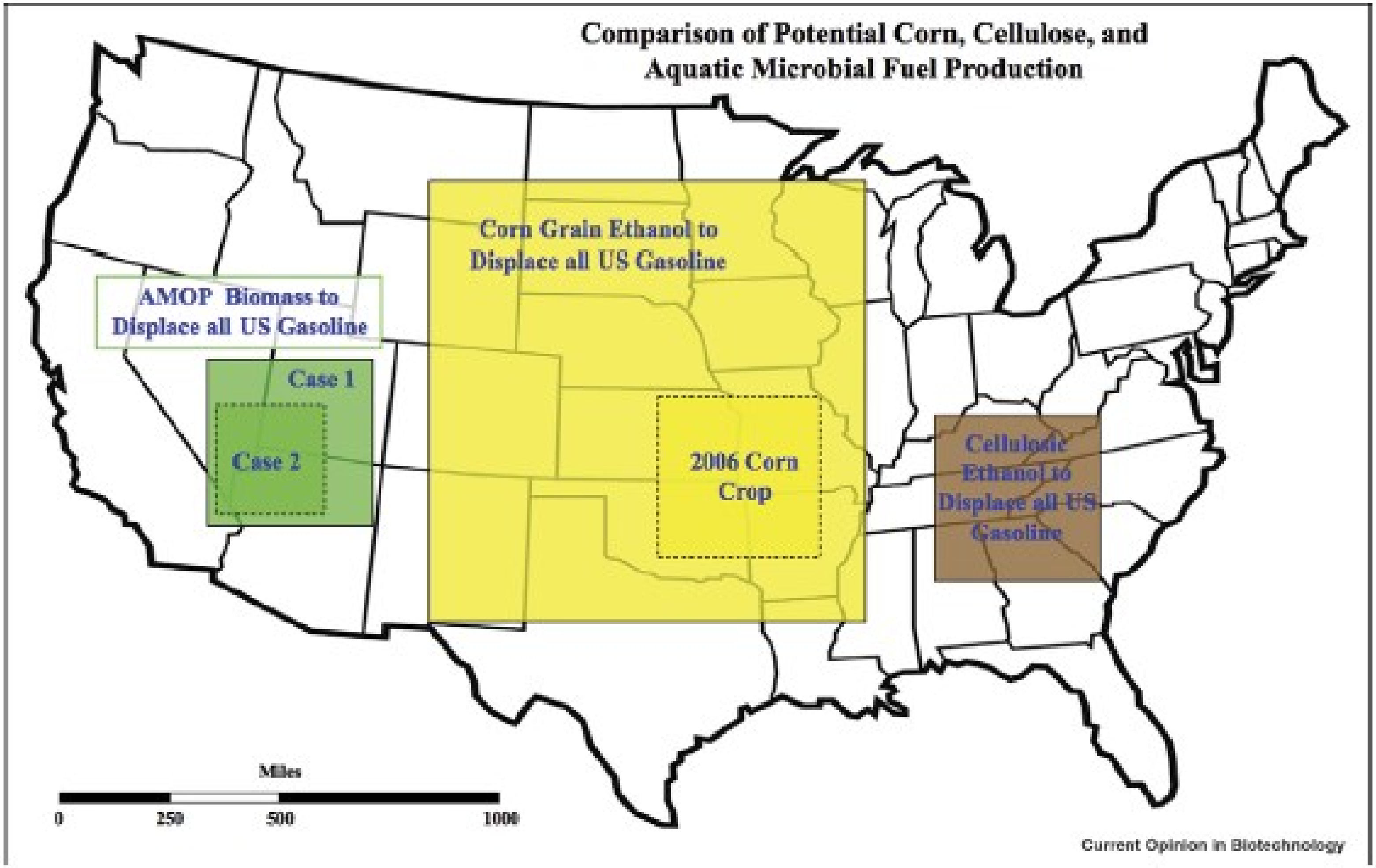
Algae do not need arable land

no competition with food production



Higher area productivity

No need of fertile land



Areas needed for cultivation of three biomass sources. Each box represents the area needed to produce a sufficient amount of biomass to convert to liquid fuel to displace all gasoline used in the USA (2006 figures) on an energy basis. Data taken from ref 24.

ALGAE vs. PLANTS

PRO Algae:

1. Superior solar energy yields

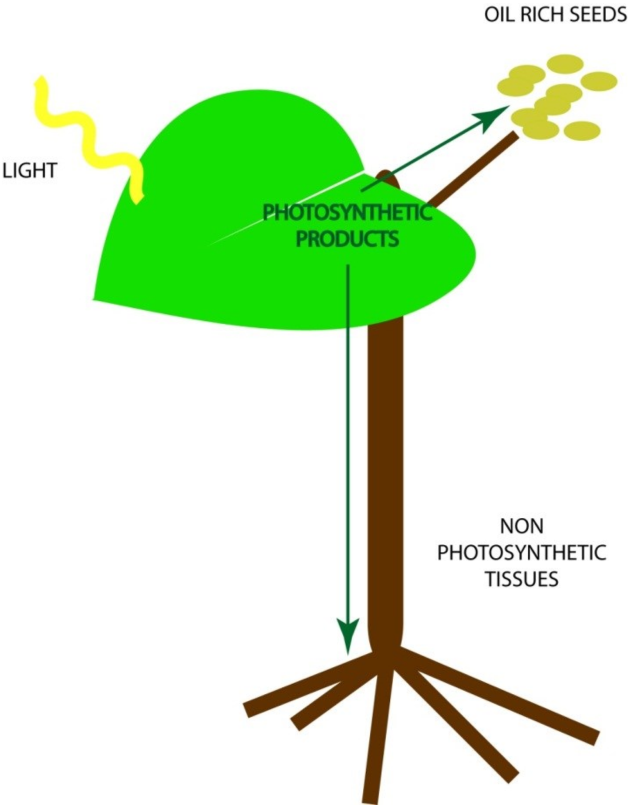
- More efficient Carbon fixation
- More efficient with high solar radiations
    - Plants experience water and thermal stress.
        - -> Photoinhibition and activation of thermal energy dissipation

- Production all year long, not seasonal

ALGAE vs. PLANTS

<u>PRO Algae:</u>

Single cells do not need to invest fixed carbon in stems and root systems



Non all tissues are photosynthetically active

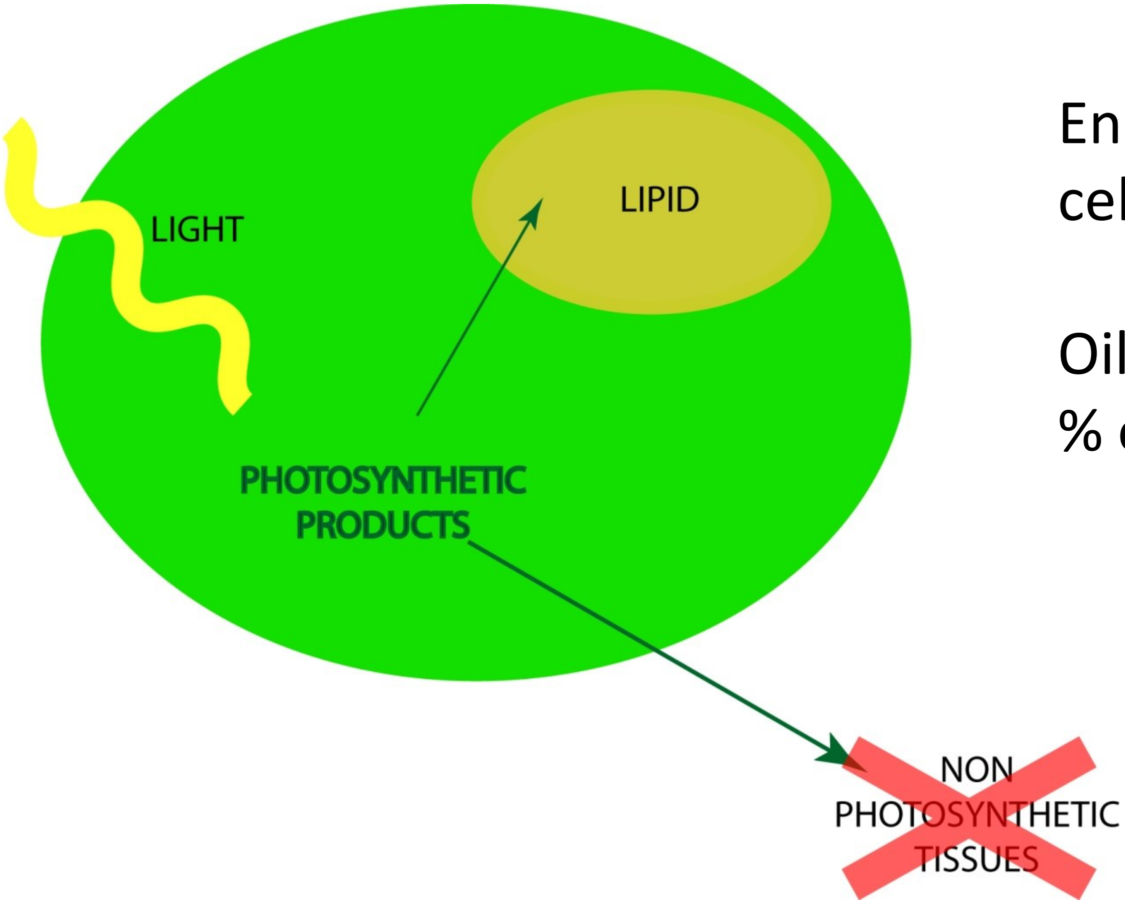
Only a fraction of the energy goes to energy storage (Oil rich seeds)

These represent ≈ 5% of total dry weight

ALGAE vs. PLANTS

PRO Algae:

Single cells do not need to invest fixed carbon in stems and root systems



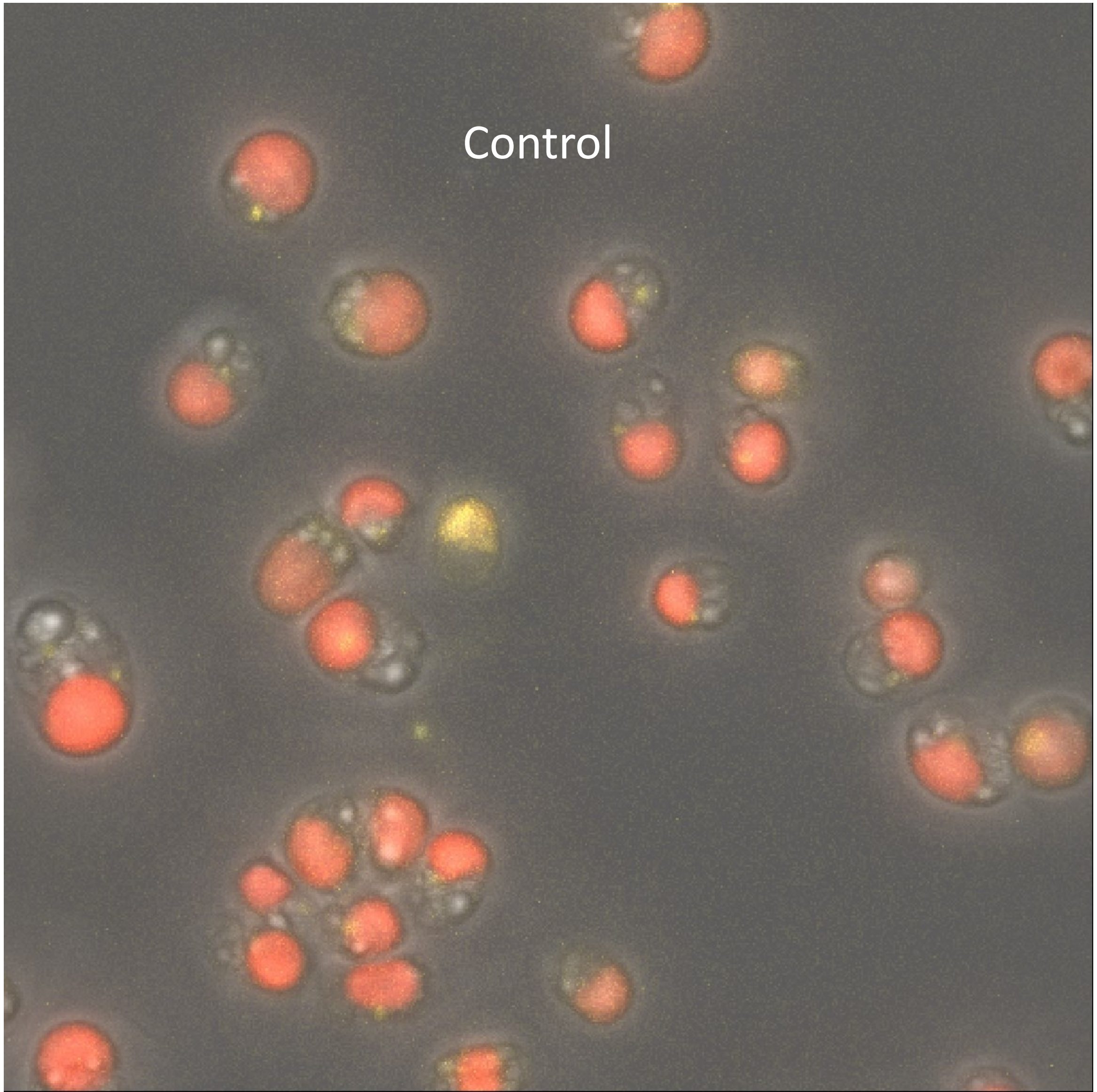
Energy is stored within the cell as lipids

Oil content can reach ≈ 40-80 % of total dry weight

Potential productivity ≈ 10 times higher

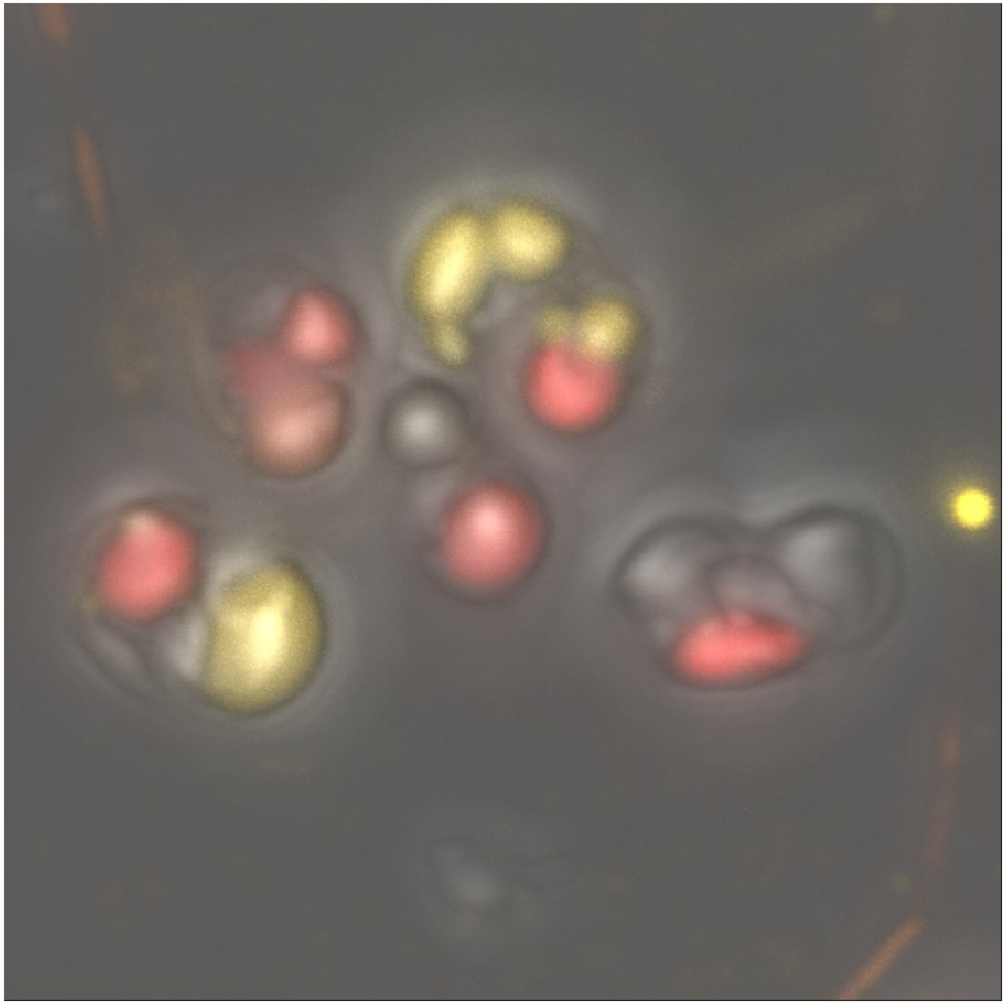
SCREENING OF DIFFERENT SPECIES

Visualization of lipid accumulation and localization by nile red staining of *Nannochloropsis*



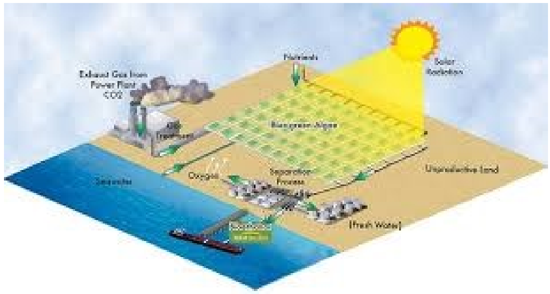
SCREENING OF DIFFERENT SPECIES

Lipid Accumulation



ALGAE vs. PLANTS

2. No competition with food production



3. Water usage

ALGAE vs. PLANTS

Algae can use salt water or wastewater

Agriculture requires freshwater

Table 5

Nutrient removal potential of consortium of native algal isolates in treated wastewater. *T1* and *T2* were the treatments bubbled with ambient air and incubated at 25 and 15 respectively. *T3* and *T4* were the treatments bubbled with 6% CO_2 enriched air and incubated at 25 and 15 °C, respectively. Ammonia-N that was 0.761 mg L^{-1} in treat wastewater on day 0 was brought to nil the next day in all four treatments.

Treatments	Days						Removal after 24 h (%)	Removal after 72 h (%)
	0	1	3	5	7	9		
Nitrate-N removal (mg L^{-1})								
T1	2.832	na	0.0097	0.0041	0.0035	0.0032	na	99.7
T2	2.832	na	0.0045	0.0039	0.0034	0.0035	na	99.8
T3	2.832	0.0073	0.0051	0.0048	0.0046	0.0043	99.7	99.8
T4	2.832	0.006	0.0045	0.0043	0.0036	0.0034	99.8	99.8
Phosphate-P removal (mg L^{-1})								
T1	4.807	na	0.0414	0.0509	0.0253	0.0149	na	99.1
T2	4.807	na	0.0576	0.0441	0.0345	0.0201	na	98.8
T3	4.807	1.1843	0.1654	0.0344	0.0213	0.0143	75.4	96.6
T4	4.807	1.128	0.1615	0.0337	0.019	0.0153	76.5	96.6

na – not analysed.

Nitrate / phosphate are important issues. Their content needs to be decresaed in wastewaters

They are instead normally limiting for algae growth

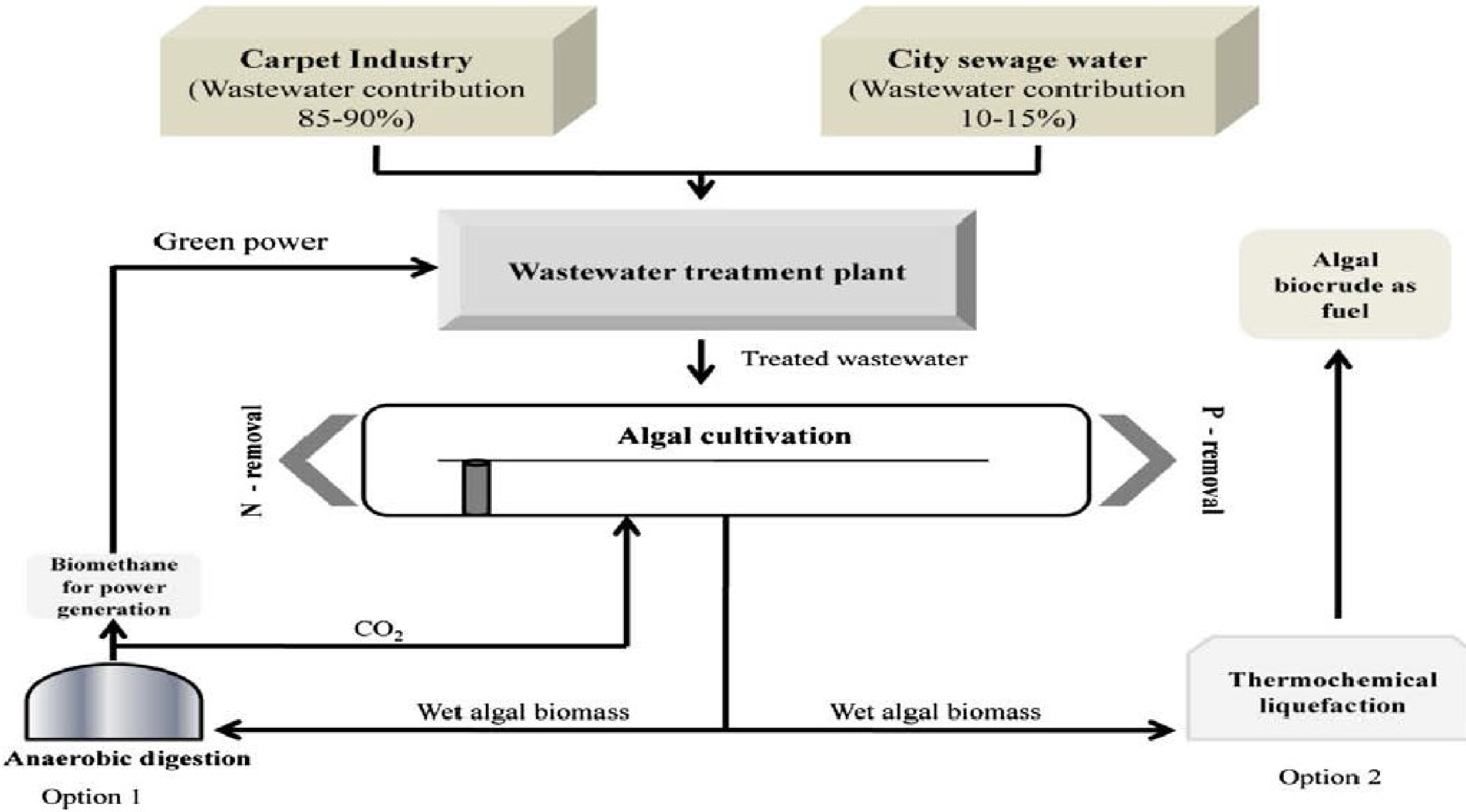


Fig. 3. Proposed scheme for biofuel production using carpet industry wastewater.

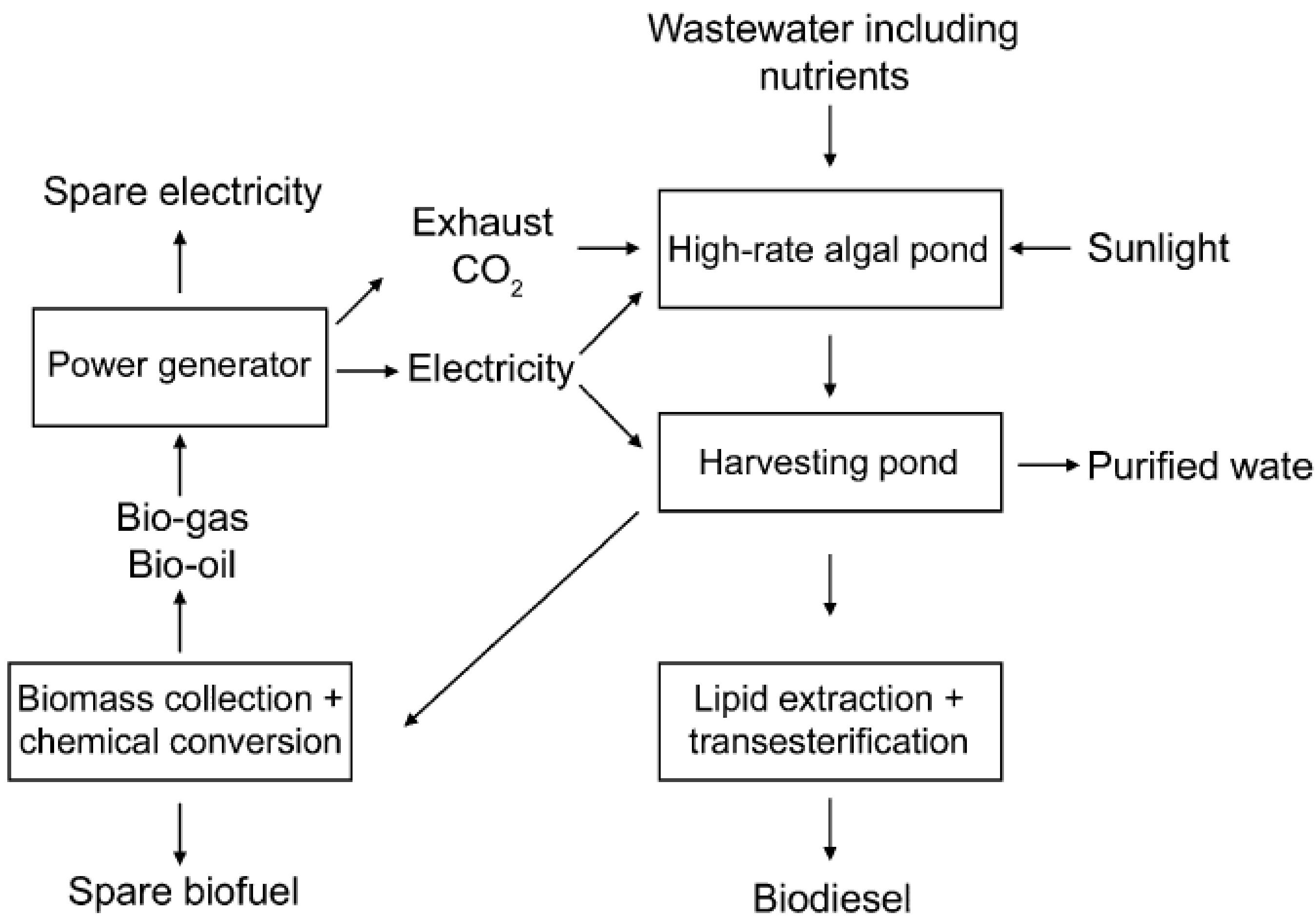
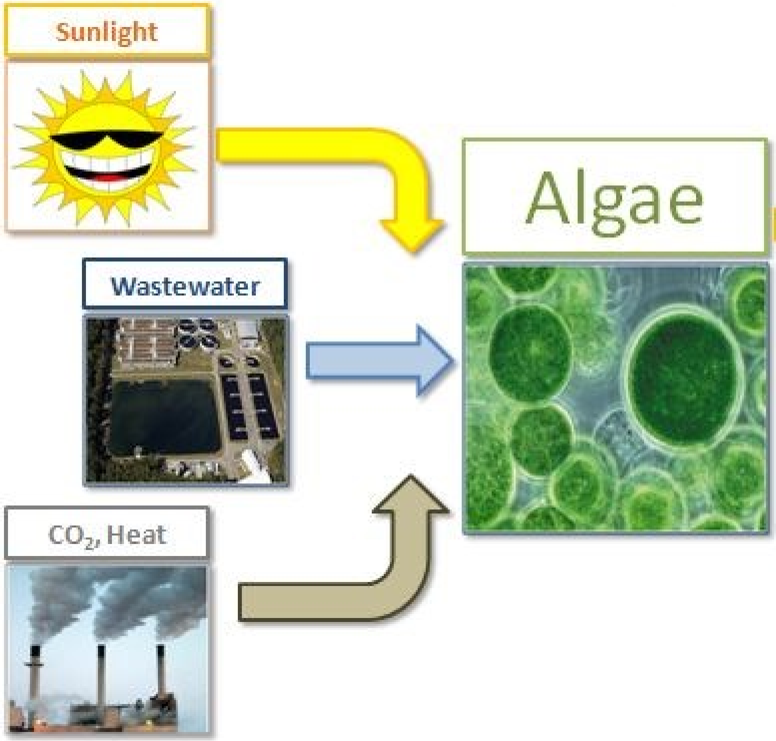


Fig. 1. A flow-diagram showing how wastewater resources could be utilised for sustainable algal-based biofuel production.

Algae can be exploited for different objectives, combined or in alternative to biofuels

Use for wastewater treatments



Particularly suitable for high N and P wastes, in combination with CO_2 producing processes

ALGAE vs. PLANTS

PRO PLANTSs:

- gas diffusion in air is 10000 times faster than in water

Algae in water are easily limiting by gas availability

->requires stirring / CO_2 supply

This is one of the main reason why productivity in natural environments is low (still annual global photosynthetic activity is due 50% to plants and 50% to algae)

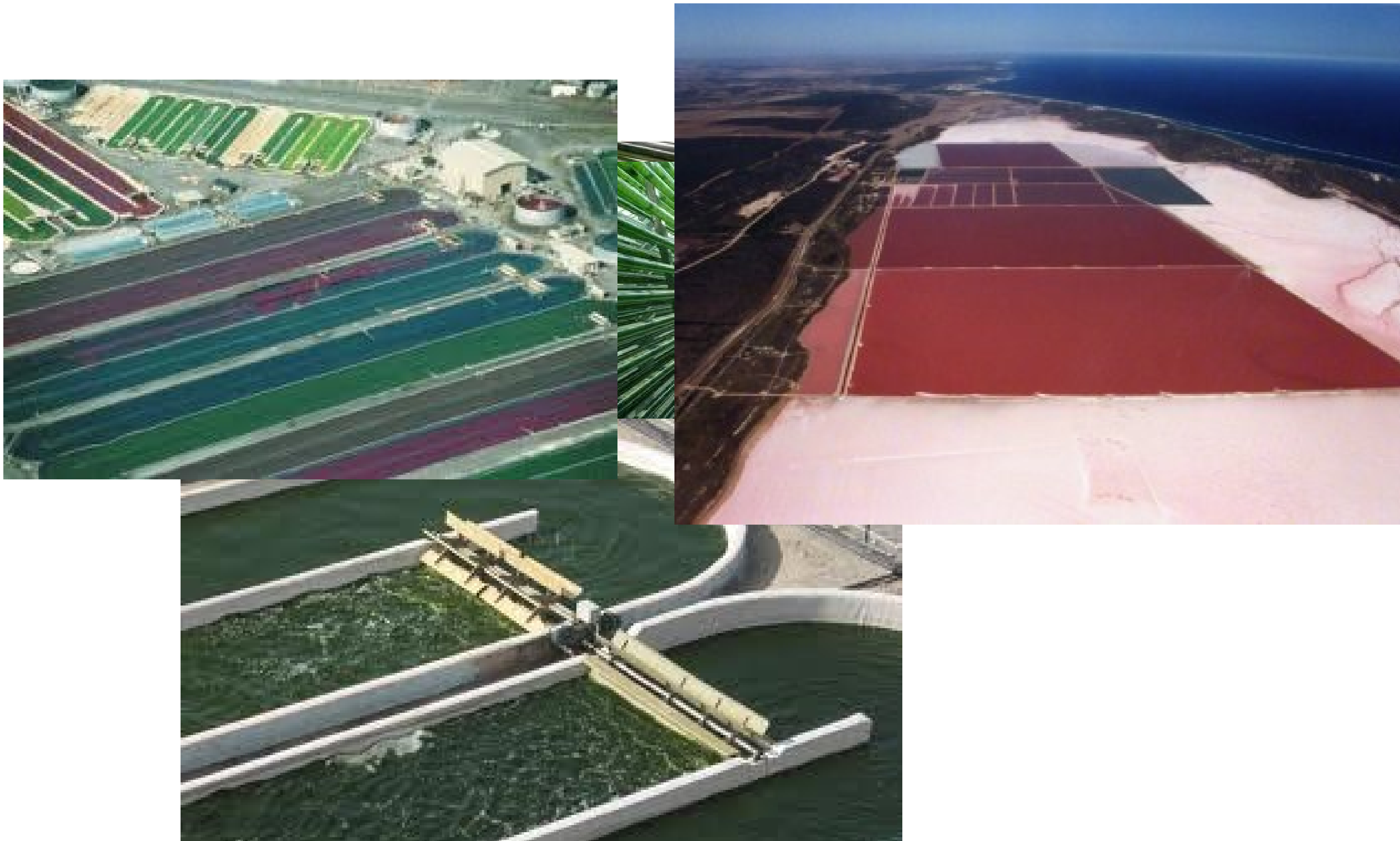
(The other is nutrient limitation, Nitrogen, phosphorous, Iron)

see algae blooms)

ALGAE vs. PLANTS

Algae requires nutrient repletion and stirring to be productive

Grown in Ponds / Photobioreactors



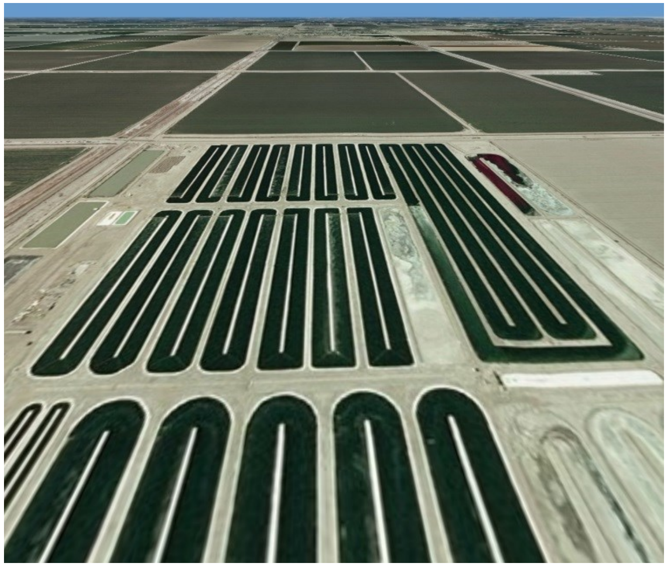
ALGAE vs. PLANTS

Algae requires nutrient repletion and stirring to be productive

Grown in Ponds / Photobioreactors

Bioreactors

Pond



Closed

Productivity is a function of the surface area rather than volume.

Bioreactors

Pond

- Cheaper
- Easy to operate
- Contamination is unavoidable
- Variable conditions
- Low energy consumption
- Low density
- Bad nutrients supply

Closed

- Expensive
- Complex (pumps, pipes, etc…)
- Much more protected
- Much more control…
- …but with a larger energy consumption.
- High density
- Better nutrient supply (CO_2)

The optimization of the bioreactors is one of the two main goals, the other one is the content of TAG.

ALGAE vs. PLANTS

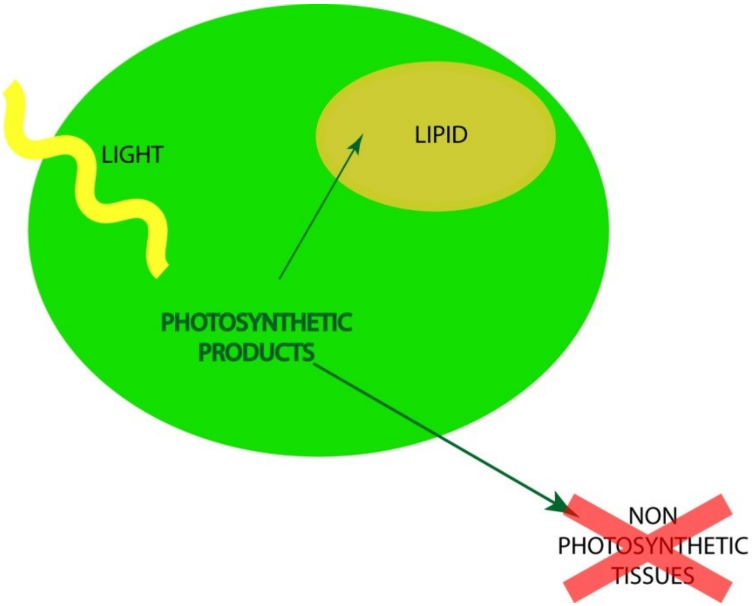
Pro plants.

problems with large scale cultivation

- Cells harvesting

A 1 g/l culture is 99.9% water

PLANTS vs. MICROALGAE



There are still several issues:

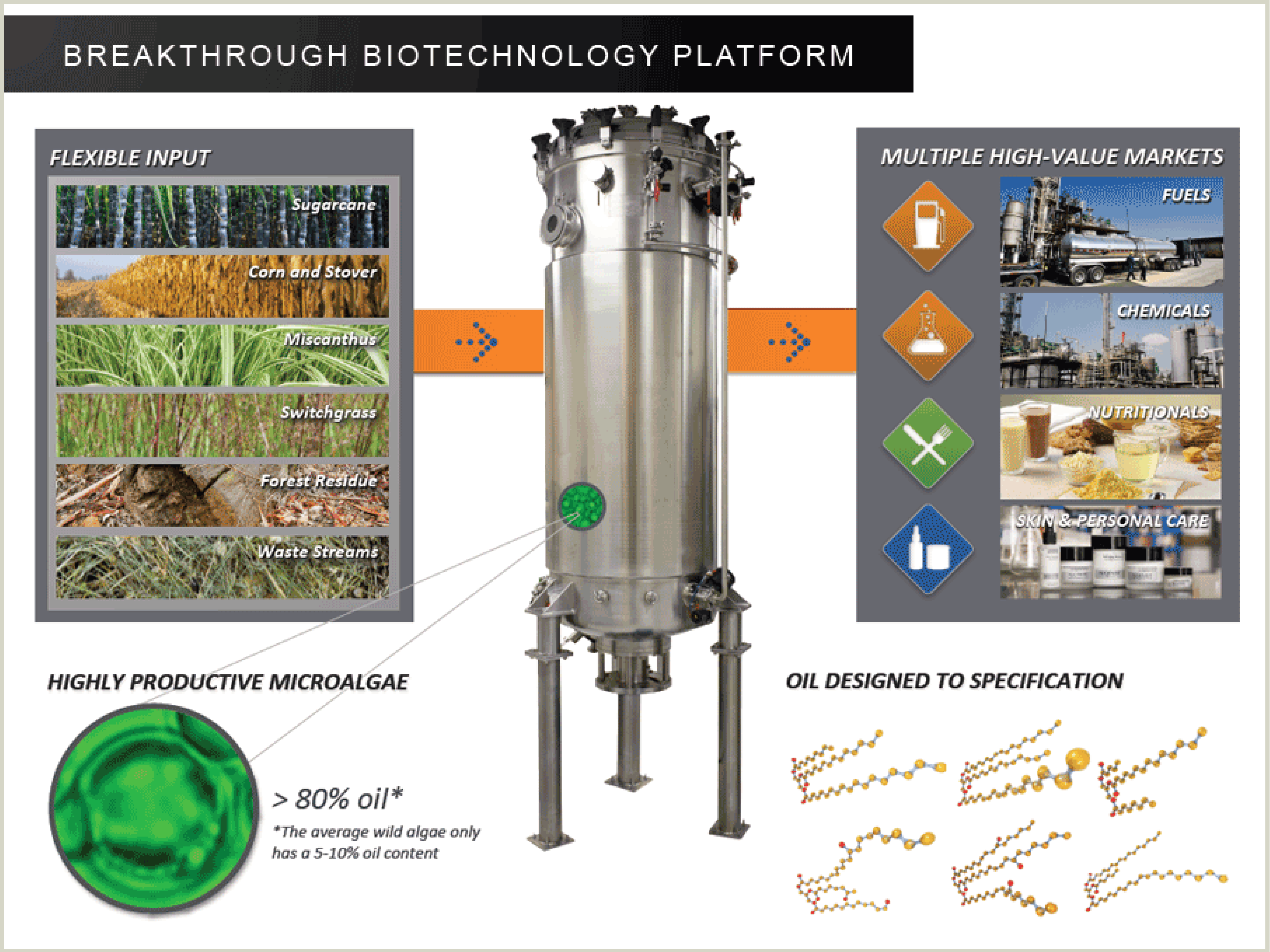
- Algae cultivation on a large scale is too expensive
- High productivities observed in laboratory conditions are not reproduced in outdoor conditions

ALGAE have good POTENTIAL, this is not a reality yet world-class molecular biology and chemical engineering capabilities, we're able to cost-effectively produce high-value tailored oils.

How the Solazyme biotechnology platform works

Most microalgae produce their own nutrients by using sunlight in a photosynthetic process. Our proprietary microalgae are heterotrophic, meaning they grow in the dark (in fermenters) by consuming sugars derived from plants that have already harnessed the sun's energy.

By using standard industrial fermentation equipment, we're able to efficiently scale and accelerate microalgae's natural oil production time to just a few days and at commercial levels.

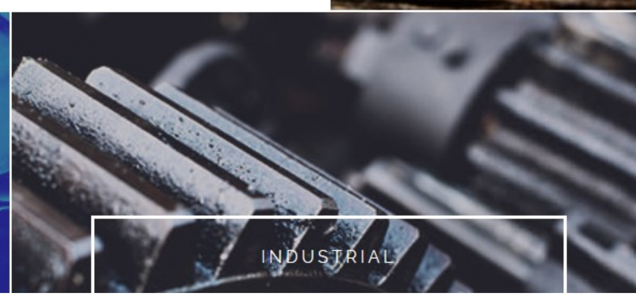


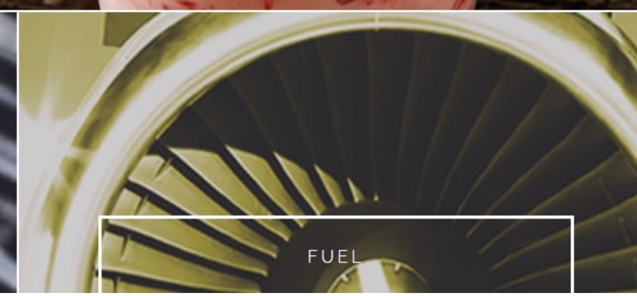
COMPANY OVERVIEW :: 2015

What We Do

Starting with microalgae, the world's original oil producer, Solazyme creates new, sustainable, high-performance products. These include renewable oils and powerhouse ingredients that serve as the foundation for healthier foods; better home, personal care and industrial products; and more sustainable fuels. Our best-in-class oils and ingredients don't just deliver long-term, sustainable alternatives to traditional sources — they can also improve the quality and performance of virtually any product formulated with them.

READ MORE





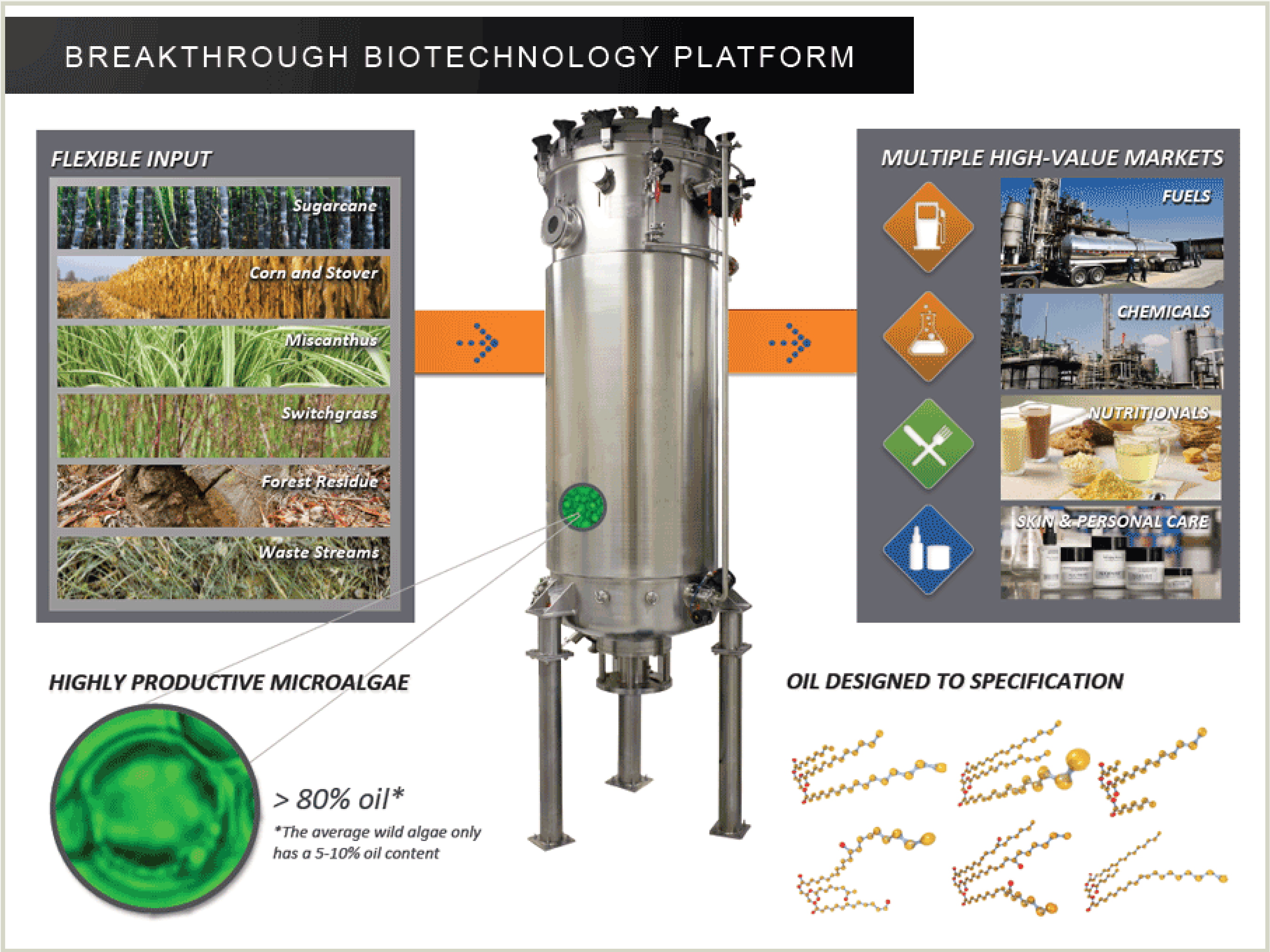
world-class molecular biology and chemical engineering capabilities, we're able to cost-effectively produce high-value tailored oils.

How the Solazyme biotechnology platform works

Biomass final Concentration – 300 mg/ml
30 % efficiency in converting sugar into biomass

…algae are heterotrophic, meaning …un's energy.

…atural oil production time to just a



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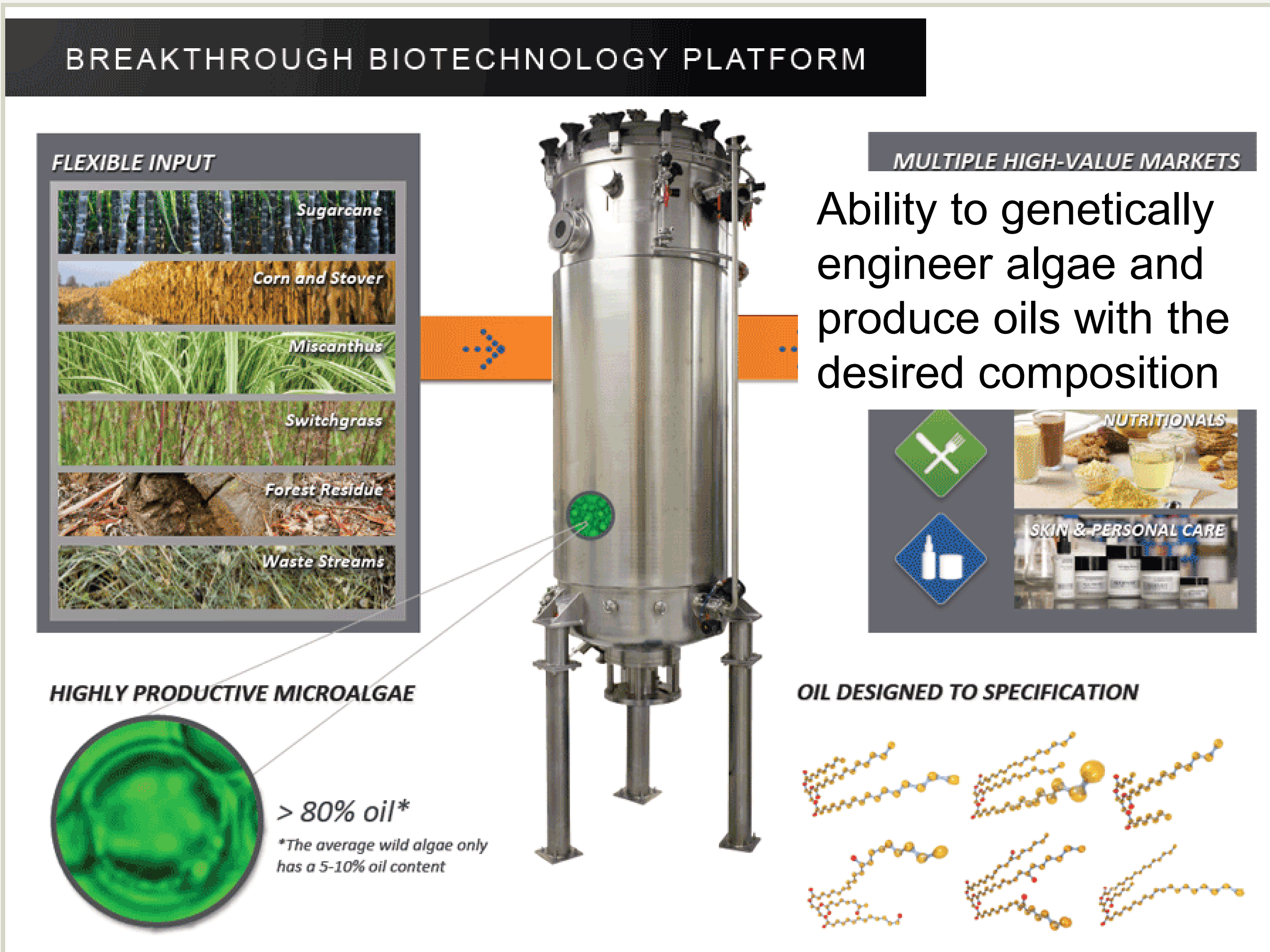


Ability to genetically engineer algae and produce oils with the desired composition

Soladiesel$_{BD}$®

A 100% algae-derived biodiesel, Soladiesel BDR can be used with factory-standard diesel engines without modification. The fuel is fully compliant with the ASTM D 6751 specifications for Fatty Acid Methyl-Esther based (FAME) fuel that meets ASTM D 975, and significantly outperforms ultra-low sulfur diesel in total THC, carbon monoxide and particulate matter tailpipe emissions. Soladiesel BD also demonstrates better cold temperature properties than any commercially available biodiesel.

Soladiesel$_{RD}$®

A 100% algae-derived renewable diesel fuel, Soladiesel RDR is a drop-in alternative to standard diesel fuels that meets ASTM D 975. Chemically indistinguishable from petroleum-based diesel, the fuel's tailpipe emissions also release fewer particulates and meet the new American Society for Testing and Materials (ASTM) standards for ultra-low sulfur diesel.

Solajet™

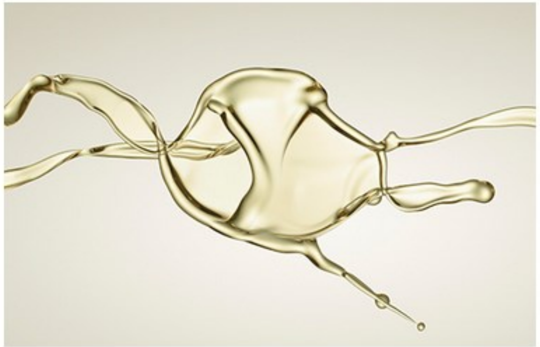
A renewable aviation fuel refined from Solazyme's algal oil, Solajet™ is the world's first microbially-derived jet fuel to meet key industry specifications for commercial aviation, ASTM D 1655. Solajet is compatible with existing infrastructure while offering key benefits, including a faster, farther and greater payload; reduced wing heat stress; lower flammability; lower smoke emissions; longer storage life; and ultimately, lower maintenance cost.

Oleochemicals

Used broadly throughout the chemical, home and personal care industries, oleochemicals are typically derived from plants or animal fats. Companies use oleochemicals to create surfactants, detergents, soaps, cosmetics, lubricants and more. Oleochemicals derived from Solazyme's unique oils and ingredients can be incorporated directly into industrial operations or used to replace or enhance existing ingredients.

Functional Fluids

Spanning a wide range of non-fuel industrial applications, functional fluids include lubricants, hydraulic fluids, solvents, drying agents and heat transfer fluids such as dielectric fluids. Functional fluids derived from Solazyme's unique oils and ingredients are valued for their tailored physical and chemical properties and low environmental impact. Download our solution to the lubricants and metalworking industry.



Encapso

The Encapso family of products offers the world's first true targeted friction inhibitors, delivering superior lubrication on demand while offering biodegradability and other environmental benefits. For Encapso sales inquiries, visit: www.encapso.com.

MARKET AREAS / CHEMICALS

Creating renewable oils for the chemical market

We're tailoring oils that can serve as the basis for the next generation of high-performance, bio-based chemicals. These oils enhance or replace petroleum-derived chemicals while improving the performance of plant oils and animal fats.

Solazyme's proprietary biotechnology enables us to create renewable, tailored oils serving a variety of chemical applications.

Our technology allows us to produce tailored oils with controlled chain lengths, saturation levels and functional group additions. The oils we produce for the chemical market can have specific melting points, varying concentrations of desired fatty acids, and high concentrations of sought-after but unusual fatty acids.

Agreements along the chemicals value chain include:

Unilever

In March 2010, Solazyme entered into a research and development agreement with Unilever to develop oil derived from algae for use in soaps and other personal care products. The agreement followed the culmination of a yearlong collaboration between Solazyme and Unilever, in which Solazyme's renewable algal oils were tested successfully in Unilever product formulations. For more information on this agreement, read here.

The Dow Chemical Company

In February 2011, Solazyme entered into a joint development agreement with The Dow Chemical Company (Dow) in connection with the development of microbe-derived dielectric fluids. Pursuant to the agreement, we began working with Dow to develop algal oils for use as dielectric fluids in the transformer market. In May 2012, we furthered entered into an offtake agreement with Dow, in which Dow agreed to purchase from us all of its requirements of non-vegetable microbe-based oils for use in dielectric fluid applications through 2015, contingent on our ability to produce such oils. We also entered into a JDA2, an exclusive, multi-year extension of our current joint-development agreement which enables additional application development work to be conducted by Dow. For more information on this agreement read here.

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AlgaVia® Whole Algae Ingredients

AlgaVia® Whole Algae Ingredients help make delicious foods that are better for people and inspire solutions for a better planet. We do that by harnessing microalgae, one of nature's first foods. AlgaVia® Proteins and Lipid Powders provide an array of benefits that can make reduced-fat foods taste richer, vegan protein fortification simpler, and the reduction of saturated fat with great taste and texture possible. Discover AlgaVia® Whole Algae Ingredients at AlgaVia.com.

AlgaWise™ Algae Oils

AlgaWise™ is a leader in next-generation food oils made from microalgae. High Stability Algae Oil offers unprecedented stability and performance with zero trans fat; Ultra Omega-9 Algae Oil is high in monounsaturated fatty acids, the good fats, so you can push the nutritional value of your formulations to the next level. Find out how AlgaWise™ Algae Oils can elevate your products tastewise, healthwise, and earthwise at AlgaWise.com

For sales inquiries,
contact: foodingredients@solazyme.com

AlgaPūr

The AlgaPūr portfolio of microalgae oils provides the purity and performance formulators in the skincare, personal care, and home care industries are looking for.These oils can deliver higher hydration levels, a silky but not greasy feel, mildness to the most sensitive skin, and improved shelf life. AlgaPūr Microalgae Oils are produced with low carbon, water and land use impact, and avoid impact on sensitive habitats, delivering better products for people and for the planet.

Algenist

The scientists behind Algenist were studying microalgae as a source of sustainable energy when they came across a revolutionary discovery: Alguronic Acid®. This compound is produced by microalgae to protect and regenerate the organism in harsh conditions. Compared to other well-known clinical skincare ingredients, Alguronic Acid shows superior anti-aging effects, including visibly reduced wrinkles and smoother, firmer, more radiant looking skin.

EverDeep

EverDeep® is a new anti-aging skincare program that transforms skin with the power of the Algasome™ complex, a rich and concentrated source of amino acids, vitamins and antioxidants. The result of 10 years of research, the Algasome complex delivers the rejuvenating essence of microalgae, minimizing the appearance of wrinkles and visibly restoring skin's strength and youthful appearance.

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MARKET AREAS / **HEALTH SCIENCES**

Improving Health through Microalgae

Solazyme's portfolio of innovative algal-derived skincare products are delivering the next-generation of breakthrough ingredients needed to help keep the skin looking healthy and young.

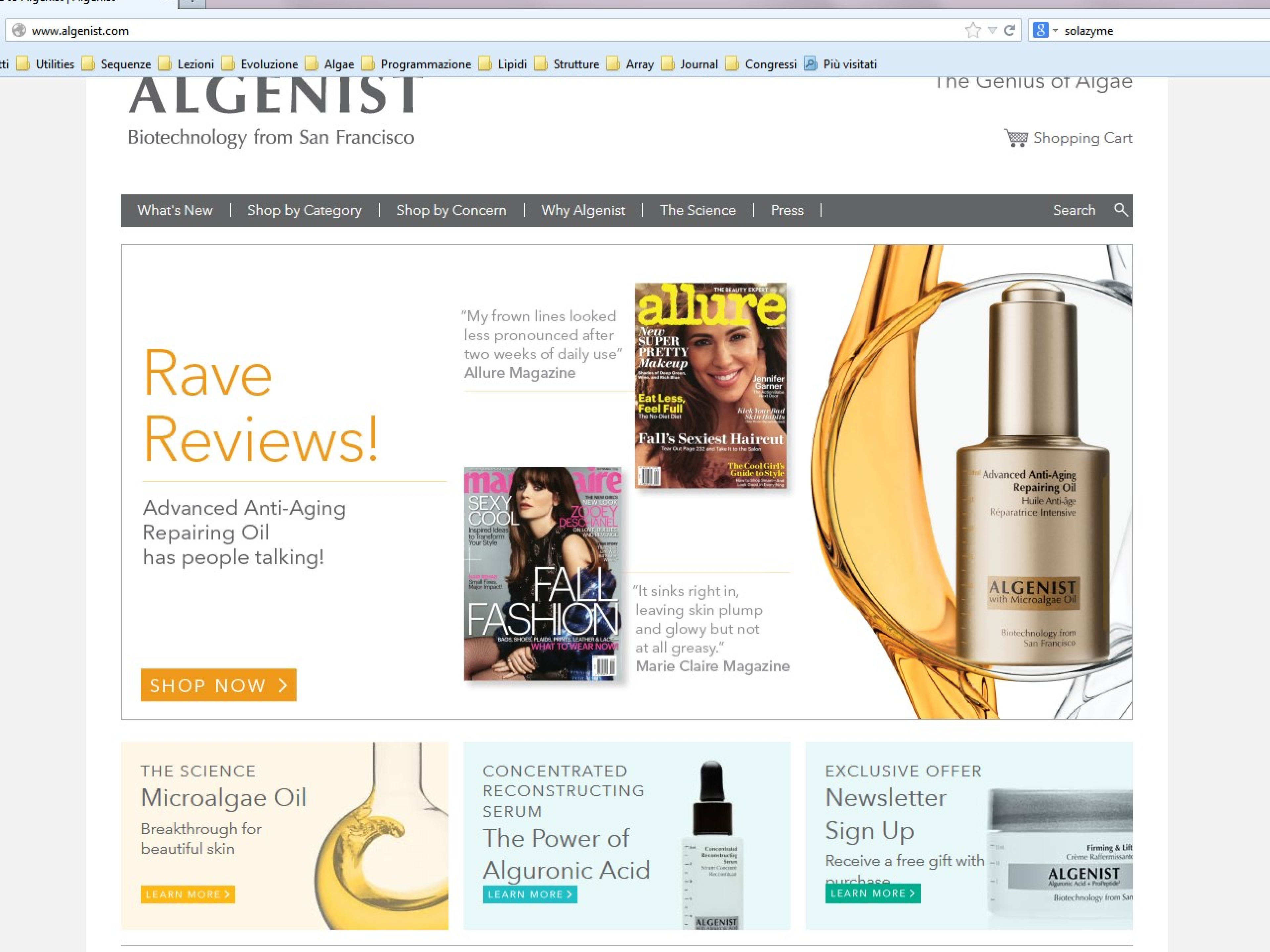
Solazyme has developed a portfolio of innovative skin care products based on the characteristics of algae, which have evolved over millions of years to protect themselves from damaging environmental factors such as desiccation and UV exposure—the same harsh elements that affect our skin.Through extensive research, Solazyme has developed algal oil based skin and personal care ingredients, including discovering and isolating a protective molecule that microalgae uses, which we've named Alguronic Acid®.

Alguronic Acid® is a unique, proprietary family of polysaccharides extracted from algae in a process we developed. When used in skin care applications, clinical and in vitro test results indicated that Alguronic Acid® delivers long-term protective benefits, as well as immediate, visible benefits.

In 2011, Solazyme signed distribution agreements of our Algenist™ skin care line with Sephora International, Sephora USA, and QVC.

To purchase Algenist online click here

ALGENIST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Biotechnology from San Francisco

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- Treatments
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SHOP BY CONCERN

Moisturizers

Algenist moisturizers offer both essential hydration and treatment benefits to the skin. Select from the range of moisturizers best suited for your skin concern.

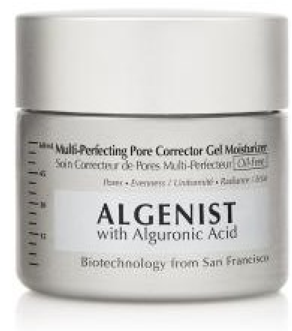
Moisturizers

SORT BY DEFAULT

Firming & Lifting Cream
$94.00

★★★★★

ADD TO CART



Multi-Perfecting Pore Corrector Gel Moisturizer
$65.00

★★★★★

ADD TO CART

Overnight Restorative Cream
$90.00

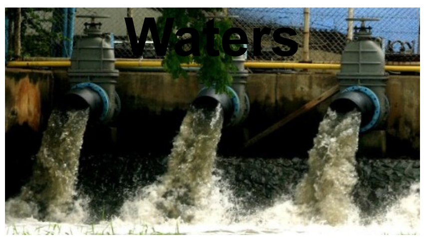
★★★★★

ADD TO CART

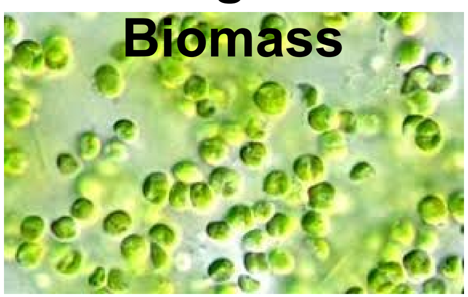
Algae exploitation for sustainable production of molecules

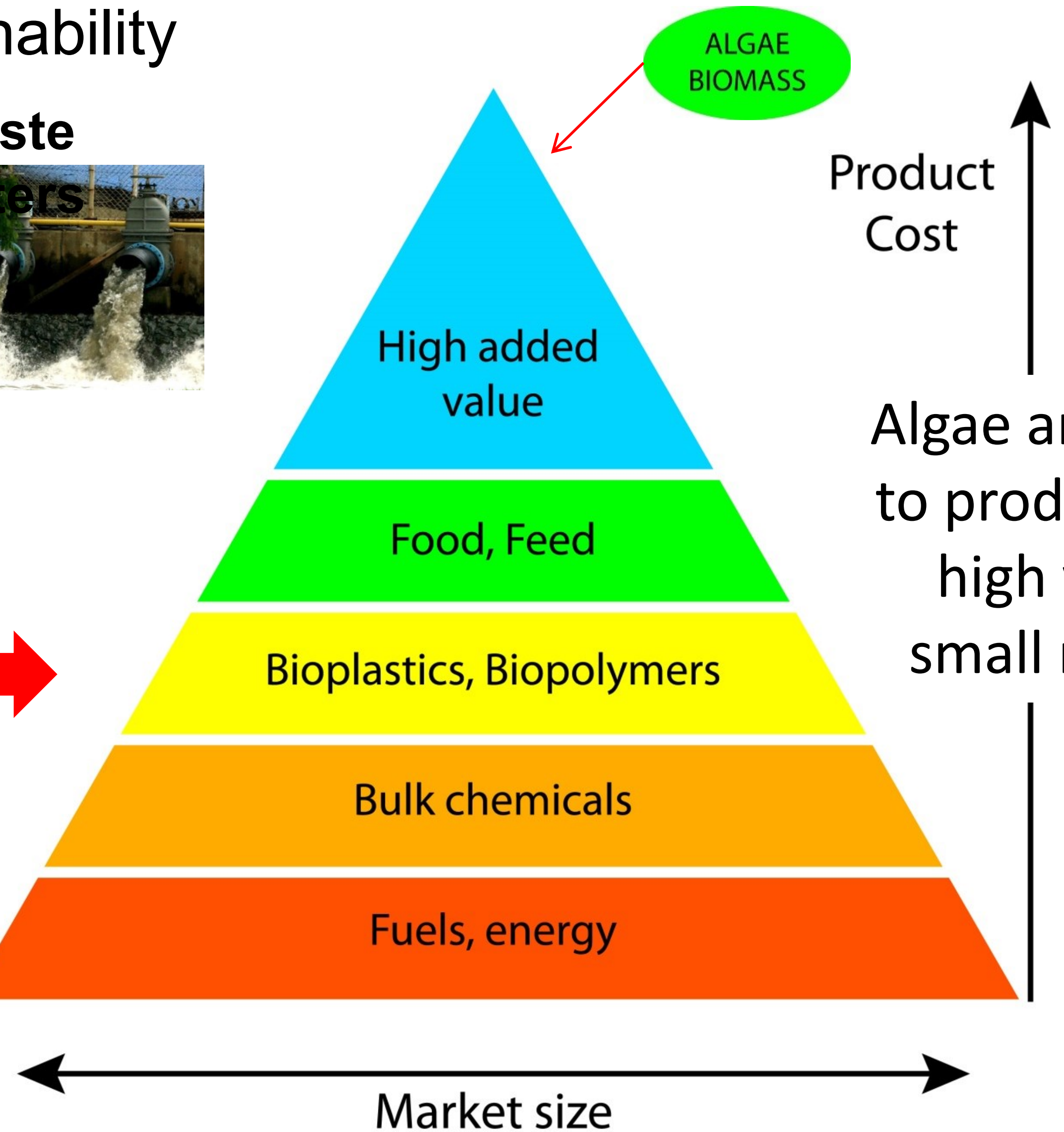
High Sustainability

Waste



Algae





Algae are limited to products with high value / small markets

Improving algae productivity is a necessity to compete in new markets