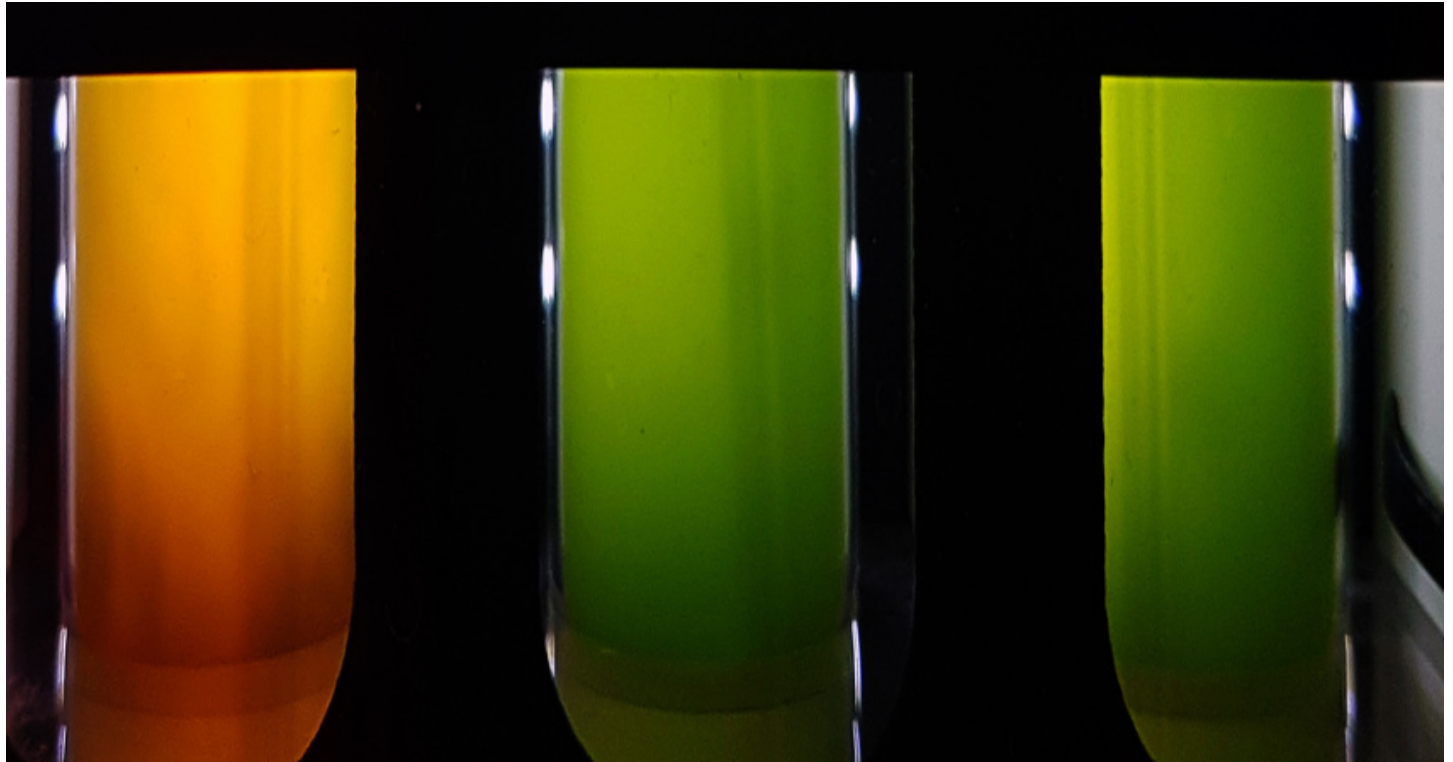


Biofuels: is the future in microalgae?

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Are we at the end of fossil fuels (coal, gas, oil) and will we know how to do without them? What alternatives are we considering? Human societies depend on hydrocarbons extracted from soil, for a multitude of applications that are sometimes ignored. There is of course the need for gasoline or kerosene for our vehicles on roads, oceans, in the air... While there are convincing solutions for running cars without gas, it is often said that the last drop of oil will be used to fly an aircraft. Fossil hydrocarbons are also a source of compounds for the chemistry – or petrochemistry – from which plastics and all kinds of materials are made, but also for the tars that cover our roads, polyurethane foams and insulation products for our buildings. What is the state of research to develop biofuels? How do we anticipate these new bio-based industrial sectors?



Figure 1. Exploitation of fossil resources. A: Coal extracted from the Kayenta mines, Arizona, USA [Photo by Peabody Energy, Inc. (CC BY 3.0), via Wikimedia Commons]; B, Oil Sands Development in Alberta, Canada [Photo by Howl Arts Collective (Flickr: tar sands, Alberta) (CC BY 2.0), via Wikimedia Commons]; C, Irish Sea Gas and Oil Production Platforms [Source: Photo by Ian Mantel (CC BY-SA 4.0), from Wikimedia Commons].

The exploitation of **coal**, **crude oil** and **natural gas** fields (Figure 1) has an environmental cost that is no longer sustainable. The exhaustion of these so-called "fossil" resources is announced, but it is still a long way off, at least several decades away. Coal has become expensive to extract in many countries, but there are still mining regions in operation. The price of oil, which also indexes the price of natural gas, is not yet dictated by scarcity or shortage and it is still possible to lower prices for geopolitical reasons. New hydrocarbon deposits are being discovered in under-explored areas such as the Arctic or offshore areas. It is possible, although expensive, to extract oil from less rich deposits such as **oil sands** (Figure 1B).

Some liquid hydrocarbons, **shale gas**, etc., seem to be possible new sources of fossil energy, but once again the environment is heavily impacted by their exploitation.

Research is therefore very active in identifying alternatives, known globally as "**biofuels**" [1]. Why? This text provides some insight, first by examining what fossil hydrocarbons are and where they come from and by evaluating the current approaches being considered for alternatives.

1. What is a fossil hydrocarbon?

The organic compounds that make up crude **oil** and **natural gas** (**hydrocarbons = carbon and hydrogen compounds**) form complex mixtures, which are fairly coarsely qualified by element contents (C, H, O, etc.) and by the average lengths of the molecules present in these mixtures (C_n = number of carbon atoms):

The **shortest carbon chains** (methane CH₄, ethane C₂H₆, propane C₃H₈ and butane C₄H₁₀) are all **gases**.

Longer chains, up to C₁₈H₃₂, are **liquid** and **chains exceeding 19 carbons** are **solid** at room temperature.

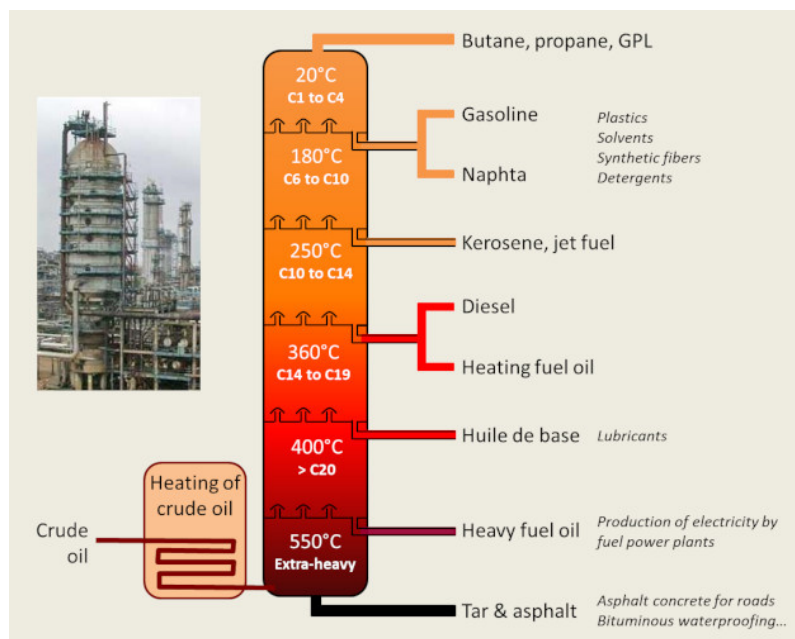


Figure 2. Representation of the operation of a petroleum distillation column. The most volatile molecules (butane gas, for example) are recovered at low temperature at the top of the column; the heaviest fractions are used for bitumen; they are recovered at the base of the column. [Source: © Eric Maréchal; Unknown photograph. [GFDL or CC-BY-SA-3.0], via Wikimedia Commons]

These molecules of different lengths can therefore be separated by a distillation process, which is the basis of crude oil refining (Figure 2). Distillation is a process for separating mixtures of liquid substances with different boiling temperatures. It allows the components of a homogeneous mixture to be separated. Chains of lengths less than C₈ are easily vaporizable, forming liquids called **naphtha**, and can be used for solvating applications (solubilizing compounds that do not dissolve in aqueous media). Chains up to C₁₂ have a lower boiling point than water.

Hydrocarbons release energy when burned, and are therefore used as "**fuels**" for all kinds of **engines**. Combustion is an exothermic chemical reaction; that is, it is accompanied by the production of energy in the form of heat. Chemical combustion reaction can only occur if three elements are combined: a fuel, an oxidizer, and an activation energy in sufficient proportion. Constraints are then imposed by the types of engines depending on the hydrocarbon to be used. [2]

Automotive fuels, for example, operate at temperatures above 100°C. For these fuels an octane number has been defined by

measuring the resistance in an engine controlled by self-ignition, *i.e.* without spark plug intervention. An **octane number** x means that the fuel behaves like a mixture of $x\%$ octane (C_8H_{18} , resistant to self-ignition), and $(100-x)\%$ heptane (C_7H_{16} , which self-ignites easily). According to this definition, the octane number of a pure C_8H_{18} solution is 100 and that of a pure C_7H_{16} solution is 0. This index is optimized for the performance of an engine, and does not reflect the amount of energy contained. In other words, a fuel is qualified according to the engine that has been developed for its combustion.

Aircraft engines, on the other hand, are subject to very different pressure and temperature conditions. "**Kerosene**" is defined in section C12-C15, followed by "**diesel**" and heavy fuels (Figure 2). The latter do not vaporize at room temperature. Chains longer than C20, solid, make up the "**paraffins**", "**tars**" and "**asphalt**" (Figure 2). There is an octane number for non-linear hydrocarbons, also containing aromatic rings. This index increases in the following order: linear long-chain alkanes < linear short-chain alkanes < alkenes (containing oxygen) and cycloalkanes (naphthenes) < branched alkanes and aromatic hydrocarbons. The properties sought for kerosenes are their purity, boiling point and low risk of explosion, low freezing point and high octane number.

Coal differs from crude oil and gas in that it is a solid form that is richer in carbon, following a transformation from lignite, coal and anthracite (three types of sedimentary rocks found in mines, which are increasingly rich in carbon). This slow process, which depends on temperature, pressure and oxidation-reduction, is called carbonization.

2. From biomass to fossil fuels

2.1. Fossil fuels are essentially of biological origin



Figure 3. Plant fossil in a Carboniferous coal piece. [Source: Photo by James St. John [CC BY 2.0], via Wikimedia Commons]

Hydrocarbons found in porous rocks and coals are derived from deposits of living organic matter, mainly plants, for about 400 million years] (see [Crude oil: evidences for its biological origin](#)). After sedimentation, the plant debris accumulated on a geological scale is gradually transformed by exposure to specific conditions of temperature, pressure, oxidation, etc., resulting in the deposits currently being exploited. The Carboniferous (about -360 to -300 million years ago) is known to be a geological stage rich in coal, and Jurassic (about -200 to -145 million years) and Cretaceous (about -145 to -65 million years) are known to be stages rich in oil fields... All these hydrocarbons burned massively every day by human activities therefore correspond to a biomass slowly accumulated over hundreds of millions of years - hundreds of millions of years ago - thanks to the photosynthesis that characterizes plants.

2.2. The energy of plant biomass comes from photosynthesis

Living matter (**biomass**) is built from organic matter (sugars, lipids, nucleotides, amino acids, etc.) that is derived entirely from **glucose** molecules produced by **photosynthesis**. In the **ecological pyramid**, organic matter enters through this sugar produced by plants, then called "**primary producers**" (see [What is biodiversity?](#)).

Two phases characterize photosynthesis ("**photo-**" for the first phase which takes place in light and "**-synthesis**" for the second phase which takes place afterwards), and allow solar energy to be captured and accumulated in organic matter.

In the **first phase**, **solar light energy** is captured, then by a process that "breaks" a water molecule by releasing oxygen (O_2), this energy is converted into **two types of energy-rich molecules**, **ATP** (adenosine triphosphate), which is the best known, and **NADPH** (nicotinamide adenine dinucleotide phosphate) which carries what is called a "reducing power".

In the **second phase** of photosynthesis, thanks to this ATP and NADPH, **CO_2 from the atmosphere is captured and reduced** (thanks to the reducing power of NADPH) to triose-phosphate (a 3-carbon phosphorylated molecule), precursor of **glucose**, a sugar of global elemental composition $C_6H_{12}O_6$.

"**Metabolism**" includes all the chemical reactions that, from this glucose, make it possible to synthesize all the molecules in living organisms. **All the organic matter, all the biomass produced in this way is rich in both carbon and energy.**

When an organism feeds on biomass, it feeds on matter but also on this solar energy, which it needs for all its biological functions. By "breaking" the material through a cellular process called "**respiration**", a process that requires O_2 , the cells **recover part of this stored energy and recycle it again into ATP and reductive power.** In doing so, breathing naturally releases CO_2 .

As a **result, photosynthesis captures solar energy and integrates it into CO_2 by synthesizing $C_6H_{12}O_6$ glucose**, and **respiration can recover some of this energy by releasing CO_2 .** The oxidation process of $C_6H_{12}O_6$, using O_2 and releasing CO_2 , is comparable to combustion, which releases energy in the form of heat. It is sometimes said that respiration "burns" sugars.

2.3. The combustion of fossil fuels, a massive source of CO_2 : a small calculation...

Considering that a tree captures CO_2 through photosynthesis and loses part of it through respiration, it can be estimated very roughly that it takes 2 kg of atmospheric CO_2 to produce 1 kg of dry plant biomass. Considering that CO_2 represents between 300 and 400 ppm (parts per million) of the atmosphere, it can be estimated that it takes about 3.5 tonnes of air to produce 1 kg of biomass. With a density between 1.1 and 1.2 kg/m^3 , it is therefore necessary to have the CO_2 present in an air volume represented by a cube of 15 m on each side to produce 1 kg of biomass.



Figure 4. Remarkable oak. It takes 2 kg of atmospheric CO_2 to produce 1 kg of the tree's biomass, but burning one kg of its dry wood releases CO_2 reaching the natural content contained in more than 3000 m^3 of air! The dry weight of this oak must be about 20 tons; its combustion will release the same amount of CO_2 as that contained in about 60 million m^3 of air! [Source: Photo by Larry D. Moore [CC BY-SA 3.0 or GFDL], from Wikimedia Commons]

The result of this simple calculation is among the most wonderful to describe the uniqueness and importance of the plant world in the biosphere. All living matter comes from this extraordinary performance of plant cells that capture an extremely minor gas from the atmosphere, CO_2 , to make it a solid that accumulates in sediments. The other side of this wonder is that when we burn 1 kg of wood, we release CO_2 reaching the natural content contained in more than 3000 m^3 (see [The Biosphere, a major geological player](#)) (Figure 4).

If it is a question of burning recently collected wood, the CO_2 emitted mostly compensates the CO_2 captured a short time ago by photosynthesis, and we can consider that this balance is neutral. But when we burn fossil fuels, we release atmospheric CO_2 into the atmosphere that had been slowly trapped for hundreds of millions of years. The atmosphere is enriched with CO_2 from the

Carboniferous, Jurassic and Cretaceous periods, which is in addition to all other industrial sources of CO₂ and contributes significantly to climate change.

3. Developing biofuels: which bio-resources, which bio-molecules?

In addition to massive CO₂ emissions, the use of fossil fuels produces various types of pollution: toxic effluents and gaseous residues produced during refining, heavy metals, carbon oxides, nitrogen oxides, soot and fine particles, etc. (see [Air Pollution](#)). Concerning crude oil derivatives, pollution by imputrescible plastics is the most visible (see [Plastic pollution at sea: the seventh continent](#)) while small molecules are also released into the environment in the form of endocrine disruptors that affect all living organisms. Emissions of polluting molecules and greenhouse gases must naturally be assessed for biofuels. Clearly, even if we are actively seeking alternatives to fossil fuels, biofuels may not solve all the questions raised.

Could we do without fossil fuels and in particular crude oil? Even if it is possible to fly a light-weight aircraft using solar energy, it is currently unthinkable to carry dozens, *a fortiori* hundreds, of passengers without kerosene. Finding alternatives also requires developing solutions to all oil derivatives, and in particular petrochemicals. The development of a chemistry that respects sustainable development and ensures the environmental balance of the environment in which it operates is called green chemistry, sustainable chemistry or renewable chemistry. However, these petrochemical alternatives do not guarantee a low environmental impact, and a major challenge that also applies to green chemistry is that it complies with environmental standards. We cannot therefore talk about “**biofuel**” without talking about “**green chemistry**”.

3.1. Bio-resources

Cultivated plants?



Figure 5. Example of crops producing sugars (sugar cane, left) or lipids (rapeseed, right) that can be used to produce biofuels. [Source: left, by Phil (Flickr: Sugar Cane 2) (CC BY 2.0), via Wikimedia Commons; right, Photo by Myrabella / Wikimedia Commons]

It was initially envisaged to simply convert part of agriculture for this need into biofuels [\[3\]](#). **Two main types of biochemical compounds are considered for this application: sugars and lipids** (see below). Agricultural production in countries such as **Brazil** has been moving in this direction, producing **sugar cane** for biofuels (Figure 5).



Figure 6. Oilseed plants suitable for biofuel production. A, *Jatropha curca*: A1, plant with fruit. Photos: A1, (CC-BY-SA-2.5); A2, by Frank Vincentz [GFDL or (CC-BY-SA-3.0)], from Wikimedia Commons; A3, biodiesel bottle made from *Jatropha* seeds, by Biswarup Ganguly [GFDL or (CC BY 3.0)] from Wikimedia Commons; B, Oil palm : B1, planting in Malaysia by Craig [Public domain], from Wikimedia Commons; B2, oil palm fruits by one Village Initiative (Jukwa Village & Palm Oil Production, Ghana) [CC BY-SA 2.0], via Wikimedia Commons; C, *Crambe abyssinica* by Kurt Stüber [1] [GFDL or (CC-BY-SA-3.0)], via Wikimedia Commons. Of these three plants, only the culture of *Crambe abyssinica* could be enduring for the environment.

Edible oilseed species such as rapeseed (Figure 5B), oil palm (Figure 6B) or lesser-known species such as camelina or jatropha (Figure 6A) have been evaluated by going as far as testing in addition to automotive fuels or kerosene for aviation. However, the need for animal and human food does not allow such a development model. The environmental cost in terms of destroyed natural areas, fertilizers, pesticides, etc., is not sustainable [4]. For example, it is now established that **it will not be possible to envisage agricultural production dedicated to biofuels in competition with agriculture for food.**

A first compromise was proposed by exploiting plants growing in non-cultivated areas, for example *Crambe abyssinica*, a mustard-like plant that uses little water or fertilizer (Figure 6C). A second highly developed track aims to exploit agricultural residues (ranging from straw or stubble, to wood waste, animal waste, crop residues, etc.). These **agricultural residues** can be produced from scratch and treated with waste, in order to extract energy from them through a **gasification** process (see below).

Microalgae and microorganisms?



Figure 7. Three-dimensional reconstruction of a diatom, *Phaeodactylum tricornutum*. [Source: photo © Denis Falconet, LPCV, CNRS Photo Library]

Since they do not compete with agricultural areas dedicated to food, microalgae, mainly **oil-yielding**, are evaluated by many research laboratories and many industrial actors. The biodiversity of microalgae is unparalleled, ranging from single-celled organisms derived from so-called “simple” endosymbiosis (**green algae, red algae**) to organisms derived from the complex assembly of several cells distant in evolution (see [Symbiosis and evolution: the origin of the eukaryotic cell](#)), following a process of “multiple” endosymbiosis (e.g. **diatoms**, Figure 7). Some of the species currently under consideration include **Chlorella** and **Scenedesmus** (green algae), **Nannochloropsis** (secondary endosymbionte).



Figure 8. Open pool used for the culture of microalgae. The water is constantly kept in motion by a motorized paddle wheel. [Source: Photo by JanB46 [CC BY-SA 3.0], from Wikimedia Commons]

To achieve a viable industry, it is also necessary to develop all the equivalent of agriculture: algae farming. It is therefore necessary to evaluate outdoor cultivation systems (open ponds, Figure 8) [\[5\], \[6\]](#) subject to climatic hazards, contamination, etc., or closed systems (photobioreactors or "PBR") [\[7\], \[8\]](#) **Growing microalgae is a water management issue**, which should therefore be considered in wastewater treatment schemes. **Growing microalgae is also a matter of managing phosphate and nitrogen inputs**, as in agriculture. It is also a question of bringing in CO₂, for example from emitting industries such as the cement industry. It is also necessary to **collect and extract molecules rich in energy**.

3.2. Bio-molecules

Sugars were initially considered because of their fermentation into ethanol (bio-ethanol) by yeasts. The simplest sugars, or fermentable sugars, from sugar cane or beet were therefore exploited (Figure 9). The bioethanol produced in this way can be mixed with fossil hydrocarbons but in a low content, as the engines are damaged by this biofuel. Bioethanol has helped to encourage the development of all biofuels because it illustrates their feasibility in concrete terms, since cars circulate every day with a share of agro-ethanol (lead-free premium fuel with 10% ethanol SP95-E10) [\[9\]](#).

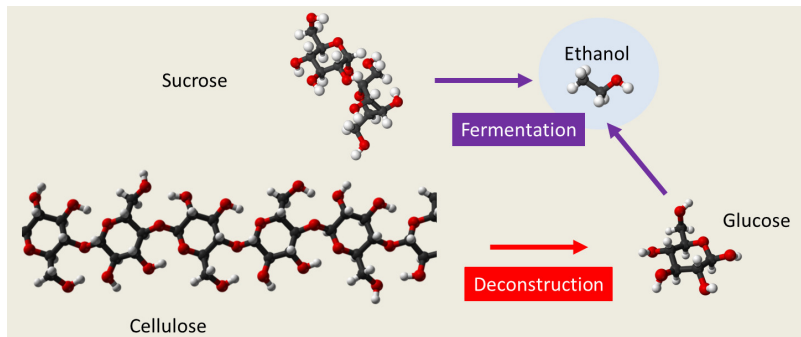


Figure 9. Three-dimensional representation of sugar-based compounds that can be used to produce biofuels: the alcoholic fermentation of sucrose (produced by sugar cane or beet) or glucose allows the production of ethanol or bioethanol. Black balls: carbon atoms; red balls: oxygen atoms; white balls: hydrogen atoms.

As indicated above, agro-resources are no longer considered viable [10]. Other sugars are actively sought: for example, those that are polymerized in the unused rigid parts of plants, **cellulose**. These sugars are assembled together, in the form of polymers, which must be undone to release the simple sugars. For this recent discipline of biochemistry, we speak of "deconstruction". These sugar polymers are associated with molecules that are very difficult to deconstruct such as **lignin**. We talk about the **lignocellulosic chain** as a new way to move towards bioethanol [11]. Microalgae are considered for molecules richer in energy and with a greater potential in green chemistry: lipids.

Lipids are the most promising class of biomolecules, on which efforts are the most important to date. We are talking about plants or **oil-yielding microalgae**. They are actually **glycerolipids**, molecules rich in long chains of carbon called fatty acids. The variable lengths of fatty acids recall the variable lengths of fossil hydrocarbons described above (in 1). The fatty acids initially produced in plants and oilseeds have lengths ranging from 14 to 18 carbons (C14 to C18), with some traces of C12, but they can also reach lengths of C20 to C24 and more. Lipids containing 3 fatty acids are called **triacylglycerols**, and form what is called **oil**.

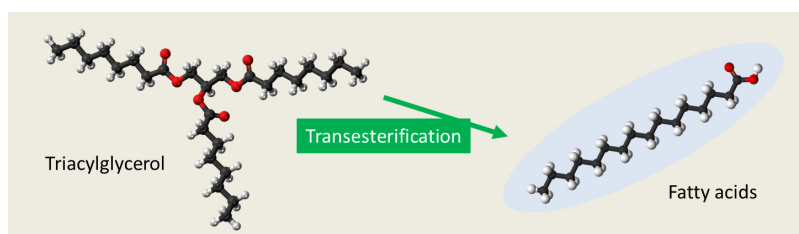


Figure 10. Triacylglycerols in algae or oilseeds release fatty acids by transesterification. These fatty acids (in fact methyl esters of fatty acids) form a biofuel close to petroleum. Black balls: carbon atoms; red balls: oxygen atoms; white balls: hydrogen atoms.

By a chemical **transesterification** reaction, it is possible to release these fatty acids, forming a bio-fuel close to oil (Figure 10) [12]. As with liquid fuels, special oil properties are required for engines, especially aircraft engines. Basically, **palm oil** is solid at room temperature and becomes fluid and liquid at high temperature, which is what is required for **bio-kerosene**. Research is therefore aimed at optimizing this type of property in oil, looking for short fatty acids and with as few double bonds as possible. However, such an oil is not always well tolerated by the plant or oil microalgae, and the biomass obtained is not sufficient. **One of the major challenges in this field is therefore to identify a biological system that produces in mass an oil that can be used as an alternative to crude oil.**

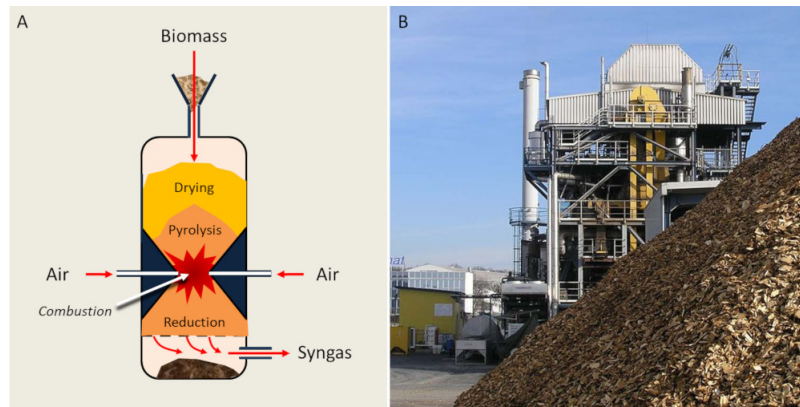


Figure 11. A. Schematic representation of a device for the production of syngas by pyrolysis from biomass. B. Biomass gasification plant in Güssing, Austria. [Source: © Eric Maréchal; right photo: Creative Commons Attribution 2.5 Generic]

Finally, **raw biomass** is also considered. In waste treatment processes, several types of conversion are considered, by biochemical, chemical or thermochemical means. This is an entire field of technological developments that are often separated from biofuels, but which is worth recalling here. A process currently being developed consists of converting dry biomass into gas, called "**syngas**", for example by pyrolysis and/or gasification (Figure 11) [13]. The composition of the syngas varies according to the biomass used and the production process. Considered to be of inferior quality, it was known at the beginning of the 20th century as manufactured gas or mains gas. Here again, the mixing of biomass with fossil coal is also considered in co-combustion processes.

There is no single, ideal solution today. In any case, it is necessary to understand how the developed agronomic or biotechnological brick fits into a larger general scheme, also to be built. We then speak of sectors.

4. Tomorrow's energy sectors coupled with agronomic, biotechnological and green chemistry sectors

The research carried out today concerns **bioresources** (with a significant effort on microalgae), **cultivation methods**, **harvesting** and **extraction processes** and finally the **conversion of extracted biomolecules** into ready-to-use **biofuels** [14].

With regard to bioresources, the question of directed evolution or production of genetically modified organisms arises. Concerning cultivation methods, the **coupling with water management systems** and **carbon-emitting industries** is being studied [15]. **Environmental impact**, input use (phosphate, nitrogen), overall **energy balance** and **sustainability** are assessed in so-called **life cycle assessments**.

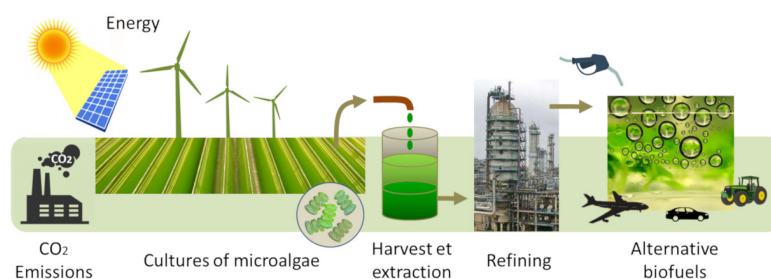


Figure 12. Process for the production of biofuels from biomass from microalgae. [Source: © Eric Maréchal]

As with any **renewable energy**, biofuel production is inefficient. **However, it has the advantage of being able to store energy in the form of biomass**, which can be integrated into the energy mix **coupled with non-storable energy production technologies**. For example, **wind turbines** produce electricity when there is wind or **photovoltaic cells** when there is sunlight, electricity that is not well stored. It is possible that this electricity could be used to illuminate microalgae culture parks, for storage that is certainly low yield, but still in the form of biomass. Today this type of coupling is considered with a treatment of microalgae by **hydrothermal liquefaction** [16].

Biomass can also be subject to **refining** [17], separating biomolecules for biofuels (e.g. oils), pigments for cosmetic or

biomedical applications, or proteins for animal production. Today, extraction systems are destructive, but this model is considered a way to reduce production costs.

In conclusion, biofuels are not yet ready to support a viable economic system [18]. The research aims to increase qualities, yields, processes, in the sense of economic viability. Given the environmental cost of fossil fuels, biofuels are not an option, but a necessity, for which the best solutions and trade-offs will have to be found. In this regard, the development of algae-based biofuels is one aspect of the implementation of algae-based solutions for agri-food, animal nutrition, cosmetics, biomedicine and green chemistry [19].

5. Messages to remember

The exploitation of coal, crude oil and natural gas fields has an environmental cost that is no longer sustainable.

Biofuels are being actively researched as possible alternatives to fossil fuels, but they are not ready to support a viable economic system.

The resources used to produce biofuels are of two types: cultivated plants (e.g. beetroot, rapeseed, sugar cane, oil palm) and microorganisms, particularly microalgae.

It is not possible to envisage agricultural production dedicated to biofuels in competition with agriculture for food.

Two main types of biochemical compounds are considered for biofuel production: sugars (for ethanol production) and lipids (for fatty acid methyl ester production).

Raw biomass can also be used to produce syngas, for example by pyrolysis and/or gasification.

Research carried out for the development of biofuels concerns bioresources (with a significant effort on microalgae), cultivation methods, harvesting and extraction processes and finally the conversion of extracted biomolecules into ready-to-use biofuels.

These systems must be designed to limit the impact on the environment, with an assessment of the overall energy balance and sustainability.

The development of algae-based biofuels is one aspect of the implementation of algae-based solutions for food, animal nutrition, cosmetics, biomedicine and green chemistry.

References and notes

Cover image. Microalgae cultures in photobioreactor (*Phaeodactylum*, in brown and *Nannochloropsis*, in green). [Source: © Photo LPCV (CEA/CNRS/UGA/INRA)]

[1] Biofuels are fuels derived from the processing of plant materials (plants, algae, etc.). When the latter are produced by agriculture (beet, rapeseed, sugar cane, sunflower, oil palm...), we also speak of agrofuels. Biofuels are considered a renewable energy source. Directive 2003/30/EC of the European Parliament and of the Council of 8 May 2003 on the promotion of the use of biofuels or other renewable fuels for transport defines biofuels as "*a liquid or gaseous fuel used for transport and produced from biomass*". Biomass being "*the biodegradable fraction of products, waste and residues from agriculture (including plant and animal substances), forestry and related industries, as well as the biodegradable fraction of industrial and municipal waste*", Official Journal No L 123 of 17/05/2003 p. 0042 - 0046.

[2] These constraints are also important for biofuels.

[3] The production of biofuels of the agrofuel type is not without environmental impact, sometimes major. The production targets set by Europe have also been reduced, with the obligation to certify production and apply sustainability criteria. While agrofuel production is part of a sustainable development trajectory, it is inherently sustainable only under certain production conditions.

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[8] Stephenson A.L., Kazamia E., Dennis J.S., Howe C.J., Scott S.A. & Smith A.G. (2010) *Life-cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors*. *Energ. Fuel*, 24:4062-4077.

[9] In September 2017, SP95-E10 volumes represented 38.5% of gasoline sold in France.

[10] The European Commission also wants to reduce the addition of bioethanol from cereals or beets to gasoline sold in Europe. France remains the leading European producer of bioethanol, with 12 million hectolitres produced each year. Some 300,000 hectares, or 1% of the useful agricultural area, provide both energy and food.

[11] Dideren I., Destain J. & Thonart P. (2009) *Le bioéthanol de seconde génération : La production d'éthanol à partir de biomasse lignocellulosique*. Presses Agronomiques Gembloux. 128 pp. (in french)

[12] Vegetable oils cannot be used as such (even when mixed in diesel fuel) to power modern diesel engines. This is why they are "esterified", i.e. transformed into fatty acid esters, by the chemical reaction of transesterification. These methyl esters of fatty acids can be obtained from: (a) vegetable oils extracted from oil plants: this is referred to as VOME (vegetable oil methyl ester), (b) animal fats: this is referred to as HOME (animal oil methyl ester) and (c) used vegetable edible oils recovered by an identified collection circuit: this is referred to as HOME (used oil methyl ester).

[13] This process should not be confused with **methanisation** applied in particular to organic waste. It allows the production of methane (or **biogas**), by anaerobic transformation of biomass using microorganisms.

[14] Delrue F., Li-Beisson Y., Setier P.-A., Sahut C., Roubaud A., Froment A.-K. Peltier G. (2013) *Comparison of various microalgae liquid biofuel production pathways based on energetic, economic and environmental criteria*. *Biores. Technol.* 136:205-212.

[15] These perspectives are the subject of research throughout the world and in particular within various European projects (INDALG, IPHYC-H2020, ALGEN, ALGAECAN, ALGAEBIOGAS, etc...).

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