

## **Biofuel production from microalgae**

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The shortage of fossil fuels is a major economic issue in the context of increasing energy demand. Renewable energies are thus gaining in importance, and microalgae-based fuels are one alternative.

### **Fuel in the world**

The fuels used on the earth can be roughly divided into two types. Fossil fuels are fuels formed by natural processes, such as anaerobic decomposition of buried dead organisms containing energy originating in ancient photosynthesis. Such organisms and their resulting fossil fuels typically have an age of millions of years, and sometimes more than 650 million years.

Fossil fuels contain high percentages of carbon and include petroleum, coal, and natural gas. In recent years, methane hydrate and shale gas have also been used. Especially in the USA, shale gas drilling technology has advanced. Another type is renewable energy, which includes biofuels.

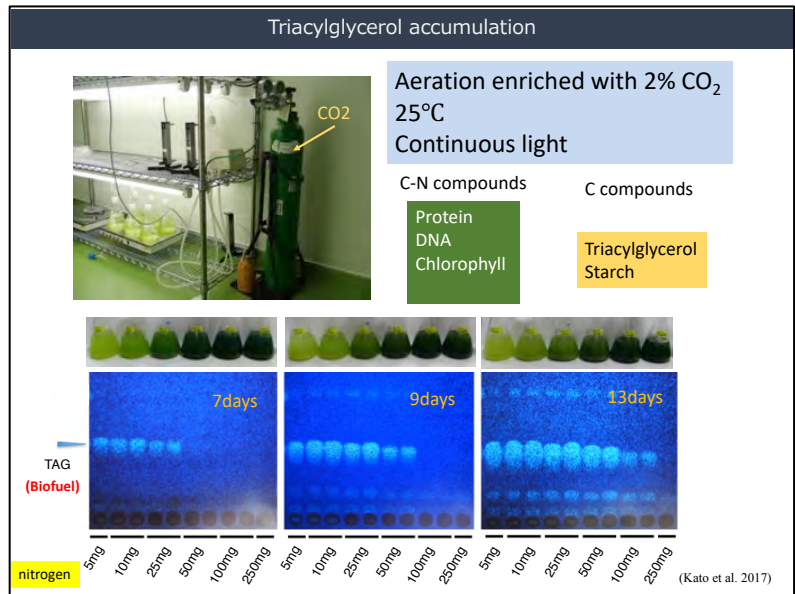
A biofuel is a fuel produced from biomass through contemporary processes, rather than a fuel produced by the very slow geological processes involved in forming fossil fuels, such as oil. The word biofuel usually refers to liquid or gaseous fuels used for transportation. The fuels produced include solid fuels such as pellets, liquid fuels such as bioethanol and BDF (biodiesel fuel), and gaseous fuels. Microalgae are attracting attention as separate sources of biodiesel.

### **Biofuel (Lipid) biosynthesis in microalgae**

Something like two-thirds of the earth's surface is covered by oceans and seas. Plants live on land. Photosynthetic plants, called algae, live in water bodies down to a depth 150 m, depending on transparency of the water. Algae occur on shores and coasts; those attached to the bottom we call macroalgae, and others live suspended in the water itself. Microalgae are microscopic algae, typically found in freshwater and marine systems, living in both the water column and sediment. They are unicellular species which exist individually, or in chains or groups.

I would like to explain about photosynthesis. Plants are called autotrophs because they can use energy from light to synthesize, or make, their own food source. Many people believe they are "feeding" a plant when they put it in soil, water it, or place it outside in the sun, but none of these things are considered food. Rather, plants use sunlight, water, and the gases in the air to make glucose, which is a form of sugar that plants need to survive. This process is called photosynthesis and is performed by all plants, algae, and even some other microorganisms. To perform photosynthesis, plants need three things: CO<sub>2</sub>, water, and sunlight. Microalgae take up CO<sub>2</sub> from the atmosphere and convert it to metabolites. Lipid accumulation is observed in some species of algae, and we can process these lipids into biofuels.

Here are some of the biofuel-producing algae we are studying. The name of our unicellular microalga is *Coccomyxa* sp. Obi, and was discovered in 2003 in Japan. This alga belongs to Trebouxiophyceae, and is close to green algae. *Coccomyxa* accumulates triacylglycerol to about 40% of dry cell weight under nitrogen-limiting conditions. Triacylglycerol is composed of glycerol and three fatty acids. Fatty acid methyl ester derived from triacylglycerol is used as biodiesel.



Here I show algae culture in our laboratory. The algae were grown photoautotrophically at 25°C in an inorganic medium with aeration enriched with 2% CO<sub>2</sub> under continuous light. Cells were cultivated at 7, 9, and 13 days at various nitrogen concentrations. The values along the bottom are the nitrogen concentrations per liter. The accumulation of triacylglycerol increased with the decrease in nitrogen concentration. Cell color changed to pale green under nitrogen starvation. The green color derives from chlorophyll. Because chlorophyll is composed of nitrogen and carbon, its biosynthesis is inhibited under nitrogen starvation. The triacylglycerol molecule contains no nitrogen atoms; and so triacylglycerol biosynthesis accelerates under nitrogen starvation.

**Biofuel and CO<sub>2</sub> emission**

Algae are aquatic species with over 3000 different breeds. They have the fastest ability to reproduce, and are more diverse than land plants. They take up CO<sub>2</sub> from the atmosphere and convert it to oxygen and can provide a high oil yield that is extracted by breaking down their cell structure. Their major advantage apart from oil mass is the ability to convert almost all the feedstock's energy into different varieties of useful biofuels. Other applications include wastewater treatment, production of energy co-generation (electricity or heat) even after the extraction of oil, CO<sub>2</sub> removal from industrial chimney gases (algae bio-fixation), bio-fertilizer, animal feed, and healthcare and food products.

Table 1  
Comparison of some sources of biodiesel

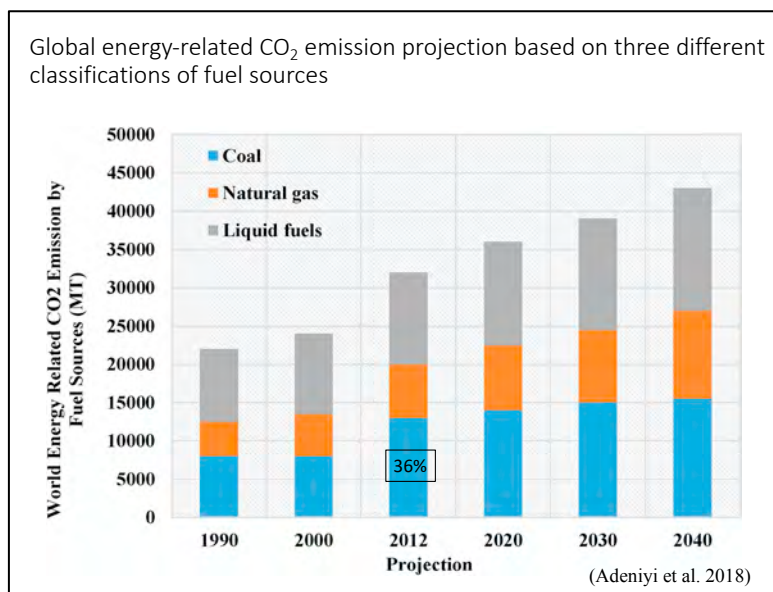
Crop	Oil yield (L/ha)	Land area needed (M ha) <sup>a</sup>	Percent of existing US cropping area <sup>a</sup>
Com	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
<u>Oil palm</u>	5950	45	<u>24</u>
<u>Microalgae</u> <sup>b</sup>	136,900	2	<u>1.1</u>
<u>Microalgae</u> <sup>c</sup>	58,700	4.5	<u>2.5</u>

<sup>a</sup> For meeting 50% of all transport fuel needs of the United States.  
<sup>b</sup> 70% oil (by wt) in biomass.  
<sup>c</sup> 30% oil (by wt) in biomass.

(Chisti 2007)

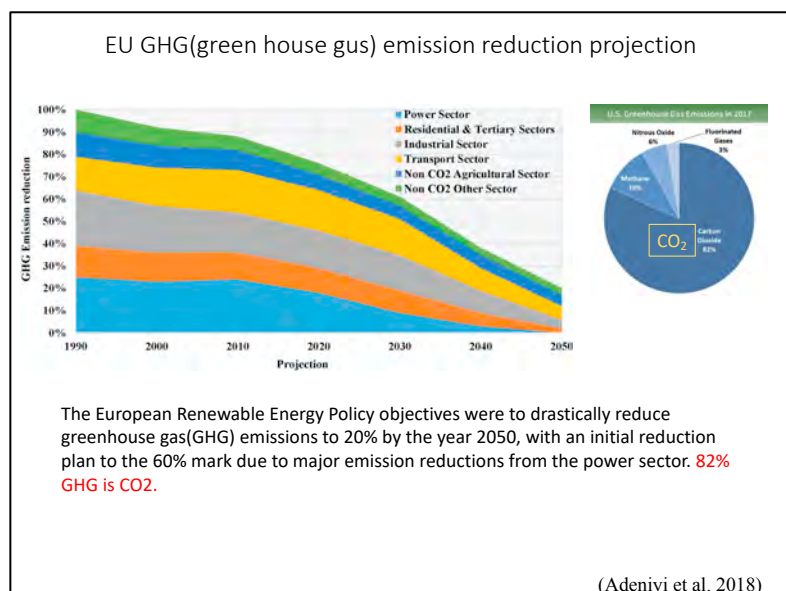
Here we compare the efficiency of algae and plant biofuel production. Using the average oil yield per hectare from various crops, the cropping area needed to meet 50% of the transport fuel needs of the USA is calculated in the right-hand column. This area is expressed as a percentage of the total cropping area of the USA.

If oil palm, a high-yielding oil crop can be grown, 24% of the total cropland will need to be devoted to its cultivation to meet only 50% of the transport fuel needs. Clearly, oil crops cannot significantly contribute to replacing petroleum-derived liquid fuels in the foreseeable future. This scenario changes dramatically if microalgae are used to produce biodiesel. Only 1 to 3% of the total USA cropping area would be sufficient to produce algal biomass that would satisfy 50% of the transport fuel needs. The microalgal oil yields given in Table 1 are based on experimental data.



CO<sub>2</sub> emissions from different fossil fuel sources have become a major threat to the environment. Although the global economy has in the past benefited from fossil fuels, they greatly contribute to the CO<sub>2</sub> level in the atmosphere, which has led to global warming. In 2012, CO<sub>2</sub> emissions generated from the consumption of liquid fuels alone represented 36% of global emissions and this is a major concern for both fuel producers and engine manufacturers.

This figure shows overall CO<sub>2</sub> emission projections as doubling by the year 2035 and might well reach 45,000 mega tonnes by 2040.



The European Renewable Energy Policy objectives were to drastically reduce greenhouse gas emissions to 20% by the year 2050, with an initial reduction plan to the 60% mark due to major emission reductions from the power sector. 82% GHG is CO<sub>2</sub>.

Algal biofuels could play a significant role in the improvement of global transport fuel and at the same time reduce the global emission of GHG. This is because transport fuels are responsible for a quarter of CO<sub>2</sub> emissions, which has resulted in the efficiency gains from biofuels as renewable fuel sources, that is often used to justify every expansion in the transport and aviation industries with respect to climate change.

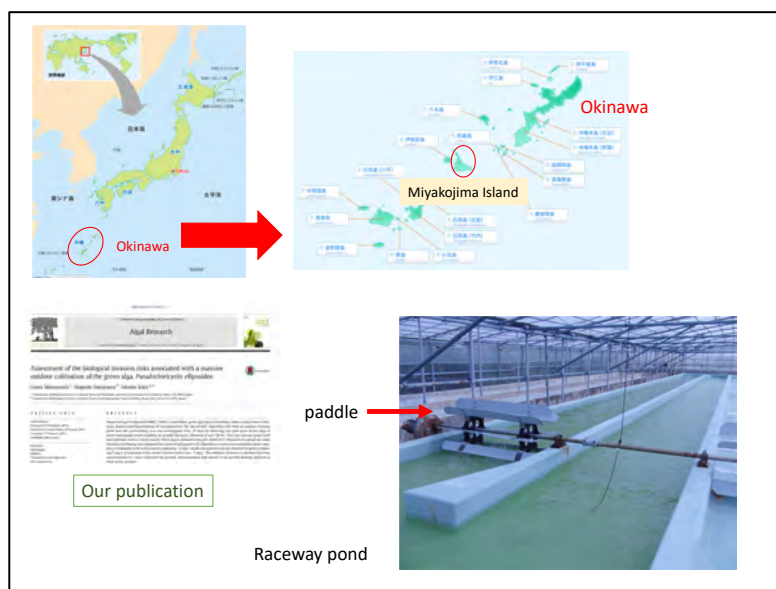
### Algal culture

Natural cultivation methods include ponds, lakes, and lagoons. These are classified based on natural water bodies: e.g. shallow, circular, and raceway ponds. These ponds are constructed in excavated pits or raised above ground level, and incorporate paddlewheels, water jets, or air pumps. Natural cultivation systems are normally cheap to construct and operate.

Artificial cultivation methods are mainly photobioreactors of different shapes such as flat plate, tubular, and column. This is a closed cultivation method for algae in a completely controlled environment, which improves productivity. However, such artificial systems are expensive.

It is possible that microalgae may inadvertently escape into the surrounding environment when cultured in large quantities outdoors. Many microalgae are resistant to drying and, because they are small, they may be scattered by wind. If microalgae leaking from a culture facility grow in large quantities in nature, there is a concern that nearby organisms and crops may be affected. However, there have been no studies on the ecological effects of the microalgae practically used as biofuels when they enter the surrounding environment, and no evaluation method has been established.

I show here an experiment to assess the invasiveness of microalgae used for biofuel production by determining the algal spread from an open raceway pond into surrounding areas.



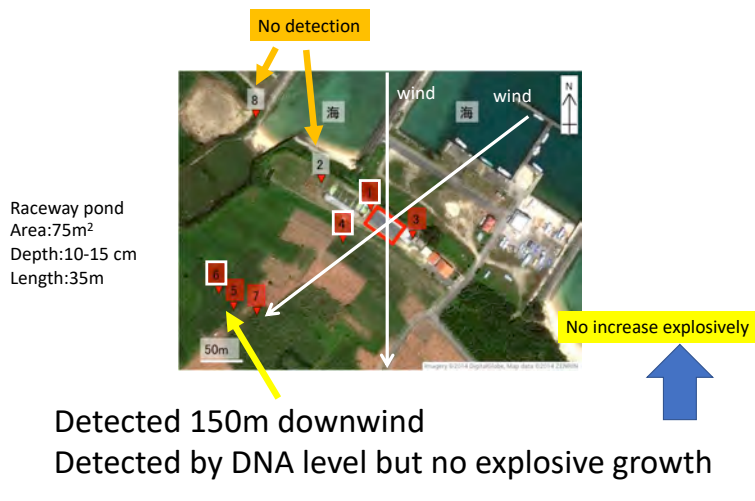
Natural cultivation methods are easy to operate, but there is a risk that they can affect the surrounding environment. Our research group has examined how much microalgae became scattered in open-cell cultures in Japan. This raceway pond is located in Miyakojima, a remote island in Okinawa Prefecture in the south of Japan. Okinawa is the place with a coral reef that was introduced in Prof. Schwarz's lecture yesterday. Being in the south, the warmth allows growing microalgae year-round.

Study area and sampling points. The red square represents the open [raceway](#) pond used for the cultivation of *Coccomyxa* sp. Obi.



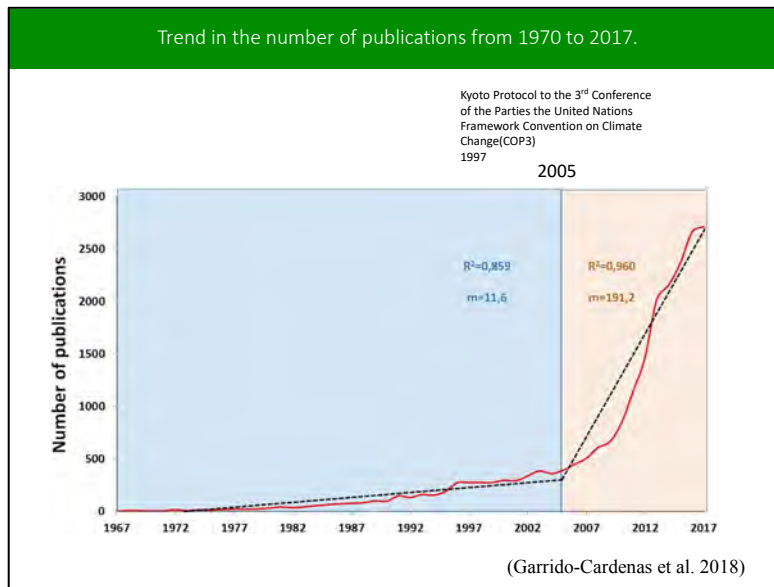
12-L buckets containing water or culture medium were set at eight places around the pond. The buckets were left for 35 days and then water samples were taken and analyzed.

Detection spread of *Coccomyxa* sp. Obi around a raceway pond



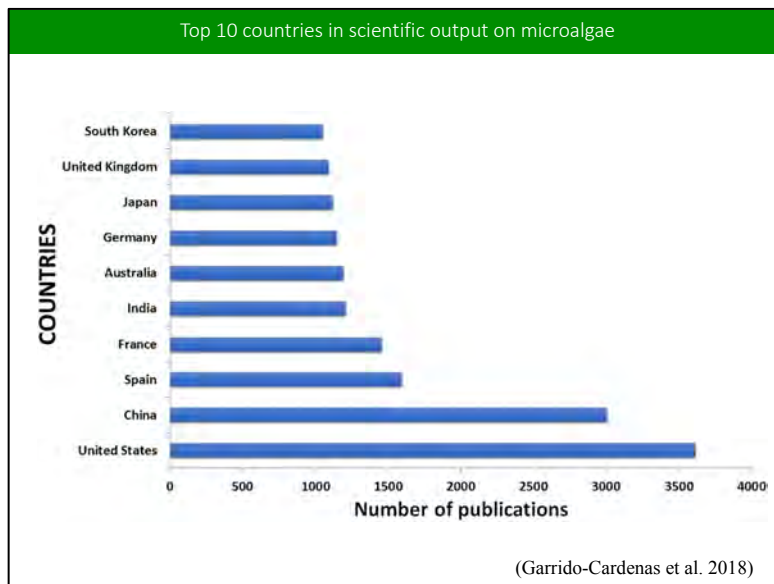
Algae from the raceway pond were detected in the bucket at the point colored red. The raceway pond was situated by the sea and was influenced by north to northeast prevailing winds during winter. The average monthly wind speeds were about 6.0 m/s. You can see the diffusion due to the wind. Also, the algae did not proliferate in large quantities in the bucket and were only detected at the DNA level. No increase explosively.

## Future prospects



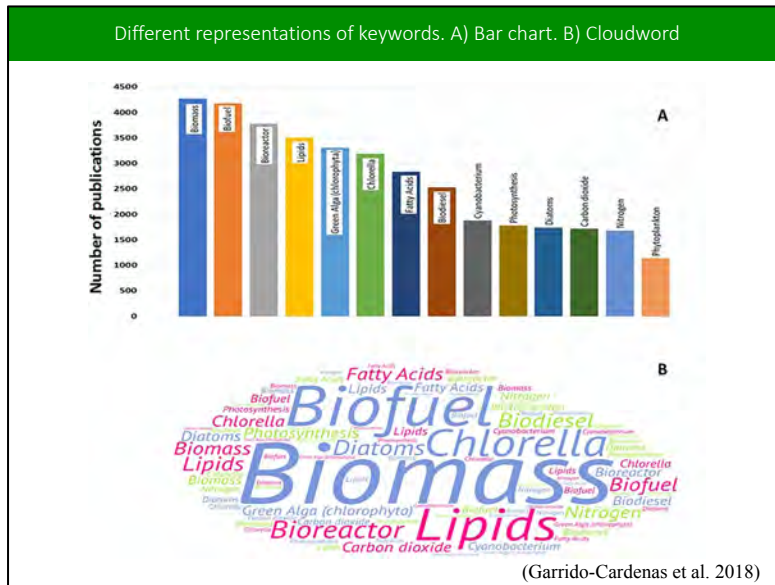
This figure shows the evolution of the number of publications from 1970 to 2017. Before 1970, there were few publications on microalgae, and 2017 is the last year for which complete data are available.

There are two clear trends over this period: the first from 1970 to 2005 and the second from 2005 until 2017. The two slope values represent the increase in the number of publications per year, showing that although interest in microalgae research increased throughout the whole period, it rose dramatically after 2005. In the last ten years, research in this field has continued to grow, reaching more than 2700 publications a year by 2017; this is a great indicator of microalgae's importance in current research.



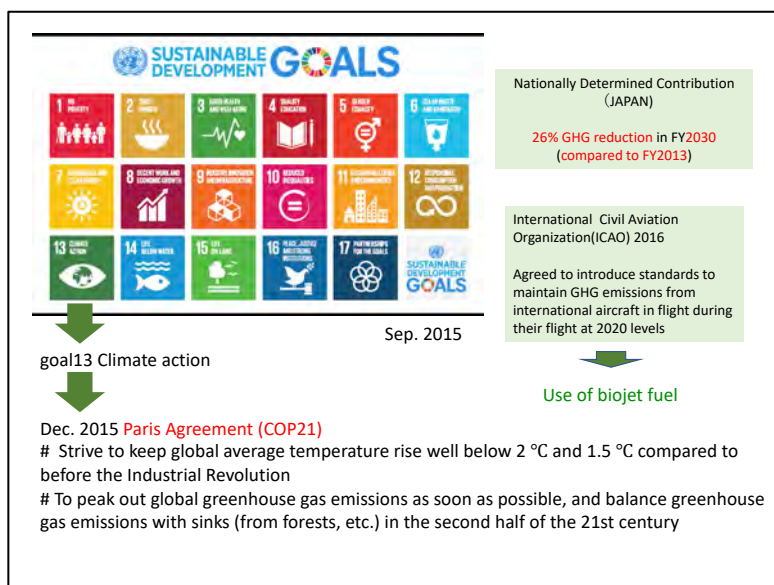
These results show the USA in first place with more than 3500 articles on microalgae over the studied period, followed by China, Spain, and then France.





For our analysis of keywords, we first discarded all those that contributed nothing to the study and/or were obvious such as ‘Microalgae’ or ‘Article’. In both cases only the keywords that appear in at least 1000 articles are represented. These mainly include terms related to the microalgae cultivation applications such as ‘Biomass’ ‘Biofuel,’ or ‘Lipids’. Others also appear that are related to the studied organism such as ‘Chlorella’ or ‘Green alga’ and some related to methodology such as ‘Bioreactor’. Of all of them, ‘Biomass’ and ‘Biofuel’ stand out as they appeared in almost 20% of publications.

The analysis of keywords in microalgae publications shows that the one with the highest presence, ‘Biomass’, is related to the first product of interest obtained from these organisms. The term with the next greatest presence is ‘Biofuel’. This is consistent with the significant interest aroused by microalgae as a bioenergetic resource; even though, to date, this endeavor has not had the expected success. Other keywords in high ranking positions relate to microalgae applications in the market such as ‘Lipids’ and ‘Fatty acids’



International goals for a sustainable and better world by 2030 were set out in the 2030 Agenda for Sustainable Development adopted at the United Nations Summit in September 2015. Goal 13 is ‘Climate action’.

The Paris Agreement was adopted on December 12, 2015, at Paris, where the 21st Conference of the Parties to the Framework Convention on Climate Change (COP21) was held. This is also a multilateral international agreement on climate change control. Here is the outline.

In response, Japan aims to reduce GHG emissions by 26% in FY2030 compared to FY2013. The International Civil Aviation Organization (ICAO) issued a statement in 2016. Japan agreed to introduce standards to maintain GHG emissions from international aircraft during their flight at 2020 levels.

Recently, Euglena, a Japanese venture company, has acquired ASTM international standards. This will enable paid flights on biofuels in Japan. Japan has increased its use of biofuels little by little. We hope that the future use of biofuels will contribute to the reduction in CO<sub>2</sub> emissions.



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