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OPINION

Can microbiology help to make aviation more sustainable?

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Abstract

Production of sustainable aviation fuels (SAFs) using microbes still requires huge research efforts to fulfill the needs of aviation, both in the biological utilization of raw materials as well as in the biological processes to convert these materials (oils, sugars, aromatic compounds and others) into SAFs. However, we should also be aware of the microbiology constraints that, in some cases, will not allow us to reach the commercial level and that, by creating false expectations we will harm the credibility of microbiologists. However, in our opinion microbiologists can and should continue to find new avenues for producing SAFs, and for evaluating the advantages and feasibility of their production. This last step will require a close collaboration between researchers and industry.

Despite the different national and international legislation and recommendations to cope with the climate crisis, levels of CO_2 are still increasing, that is from 387 ppm in September 2008 to 419 ppm in September 2022 (https://climate.nasa.gov/vital-signs/carbon-dioxide/). In the transportation sector, in which most of the greenhouse gas (GHG) emitted is CO_2, emissions have increased from 4.6 billion tons in 1990 to 8.2 billion tons in 2019 (https://ourworldindata.org/), representing approximately 16% of the total emissions. In 2020, international aviation emitted around 2% of the total net emission of GHG, with 55.879 kt CO_2 eq. Although these are the latest data released, an increase is expected in the coming years, because, although in 2020 the COVID pandemic had limited travel, it is estimated that air transportation will be 10 billion travellers in 2050. At the 77th International Air Transport Association (IATA) Annual General Meeting on 4 October 2021, the IATA member airlines signed the agreement of achieving net-zero carbon emissions from their operations by 2050 (https://www.iata.org/en/pressroom/2021-releases/2021-10-04-03/). This means that at least 1.8 gigatons of carbon must be reduced by that year (a total of 21.2 gigatons from now to 2050). This will require a tremendous and coordinated effort for the industry, governments, scientists and engineers. Governments will have to provide policies and financial incentives for infrastructure providers, the industry will have to take risks to test and to implement new technologies, and scientists and engineers will have to conceive, develop and design new inventions and provide them to the companies.

IATA has designed a plan, in which 65% of the emissions will be reduced through the utilization of sustainable jet fuel, 13% by the utilization of new propulsion technology (hydrogen), and 3% through efficiency improvements. Carbon capture and storage and credible offsetting schemes will account for the rest of the emissions. However, as indicated by Kallbekken and Victor (2022), sustainable fuels are not advancing as quickly as expected and there are other environmental concerns related to air aviation besides CO_2 emissions. Therefore, other approaches, such as the design and construction of new propulsion systems, adjustments of flight schedules and itineraries to select the most favourable atmospheric conditions and better organization of terrestrial operations to achieve maximum efficiency, should contribute to coping with the problem (Kallbekken & Victor, 2022).

Sustainable aviation fuels (SAFs) are defined as fuels with the potential to generate lower carbon emissions than conventional fuels during their whole life

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cycle, from origin to combustion. Conventional jet fuel is based on kerosene (Jet A and Jet A-1) or gasoline (Jet B). Jet B is more inflammable and difficult to operate than Jet A and, therefore, most jet fuels are based on kerosene that is a mixture of alkanes (linear, branched and cycloalkanes), olefins and aromatic hydrocarbons, with alkanes being the most abundant compounds. So far, certified SAFs should be mixed with conventional aviation fuels at a maximum blend ratio of 50% without requiring the adaptation of aircraft or engines, however, the future use of 100% SAFs by 2030 is under study. The International Civil Aviation Organization (ICAO) webpage (https://www.icao.int/) periodically reports about agreements between SAF producers and aviation companies to supply SAFs (i.e. SINOPEC with Airbus, OMV with Ryanair, Air Company with JetBlue and Virgin Atlantic), trends in environmental issues related with air transportation and also includes a worldwide map of present and planned SAF facilities. However, most of the SAF facilities are not in service yet and improvements will have to be made to adjust the capacity to produce SAFs in the near future with the proposed goals.

There are several technologies for the production of biofuels, depending on the starting substrate; gas-to-jet (GTJ), alcohol-to-jet (ATJ), sugar-to-jet (STJ) and oil-to-jet (OTJ) (reviewed by Jiménez-Díaz et al., 2017) (Figure 1). Microbes are involved in all the technologies, although at different steps. In GTJ, gasification of biomass under a limited supply of oxygen produces *syngas* which is a mixture of carbon monoxide, carbon dioxide, methane and hydrogen. In general, biomass is treated at a high temperature to produce syngas that is later transformed, via Fischer–Tropsch synthesis, into liquid hydrocarbons. However, Fisher–Tropsch catalysis uses high temperatures and pressure requiring high energy inputs; for these reasons, biological alternatives have been considered. Syngas fermentation by chemoautotroph microbes, such as *Clostridium*, have been successfully used to convert CO-rich waste gases into short- and medium-chain alcohols (Phillips et al., 2017). The challenge to scale up this process is related to the inhibition of the microbial growth or of crucial enzymatic activities by gas impurities (such as NH_3, H_2S and NO_x). These short- and medium-chain alcohols, produced from syngas, have to be chemically converted to jet-fuel. Therefore, the ATJ technologies consist of the biological production of alcohols that should be chemically dehydrated, oligomerized and hydrogenated to produce SAFs. Jet fuels produced from alcohol have been approved to be mixed with conventional jet fuels and companies are improving their technologies to produce ATJ SAFs in the near future. For example, Lanzajet is expecting to produce 9 million gallons of ATJ SAFs annually in 2050.

Several strategies to convert sugar to fuel, including microbial isoprenoids and sesquiterpene production, utilization of the cyanobacterial alkane biosynthetic pathway or the engineered reversal of the β-oxidation cycle for the synthesis of fuels were explored a decade ago (reviewed and referenced in Jiménez-Díaz et al., 2017) (Figure 1). In most cases, genetically engineered, well-known bacteria or yeast were used to produce these compounds. However, whatever pathway or micro-organisms have been used for the conversion of sugars into jet-fuels, titre, yield and productivity have

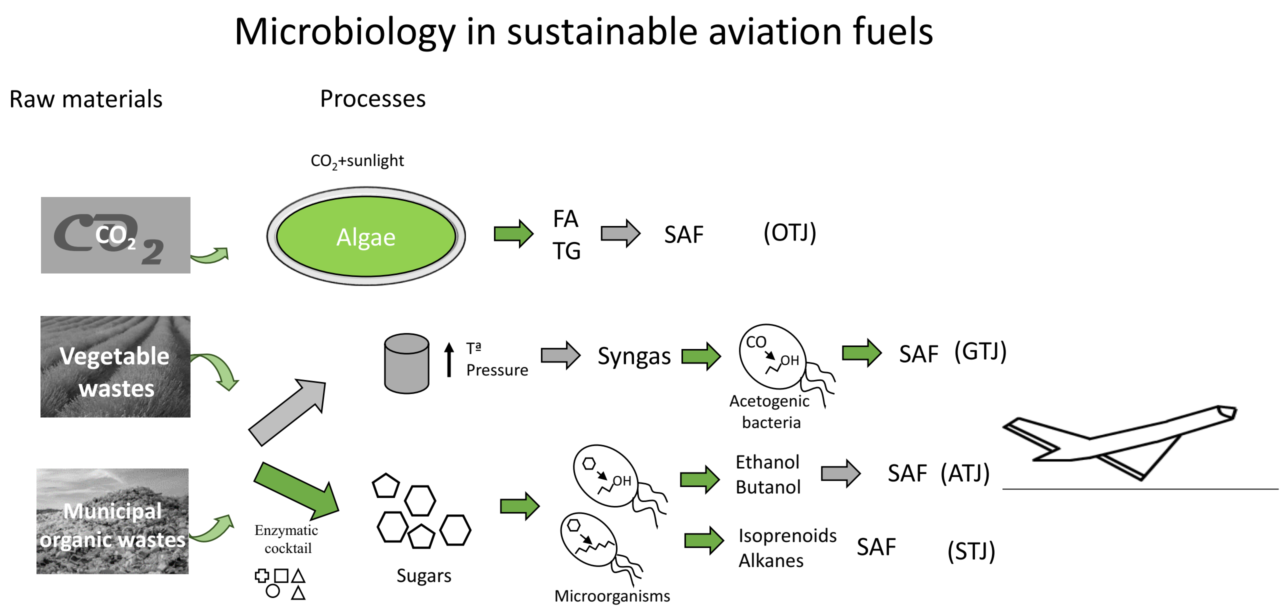

FIGURE 1 Schematic representation of the sustainable aviation fuel (SAF) production processes in which microbiology is involved. The green arrows indicate biological processes, whilst the grey arrows indicate physico-chemical process. Downstream processes to purify the compounds to finally obtain SAF are not shown.

to be improved to reach not only profitable production (which only in some cases has been achieved) but to scale up the process so as to be a global solution for aviation. Microbial metabolic pathways are tightly regulated in both native and recombinant micro-organisms. Therefore, directing the metabolic fluxes towards the desired product will require not only the introduction and correct expression of the necessary metabolic genes, but also the modulation of the appropriate levels of cofactors, and the correct expression of efficient energy systems. Improvement of our comprehension of the microbial metabolic fluxes and their regulation, identification of the bottlenecks in synthetic pathways and advances in enzyme engineering to optimize these key enzymatic steps, will be part of the research needed to improve jet-fuel production from sugars. Sugars, suitable for SAFs, should be obtained from biological materials. The first generation of bio-fuels, which used seeds or edible crops for fuel production, raised the controversy of using arable land for "fuel vs. food" production. Therefore, scientists and engineers started working on the second generation of bio-fuels, those that used non-edible biomass as raw materials. Utilization of cellulose and hemicellulose from plant material presented the challenge of using highly recalcitrant polymers (cellulose, hemicellulose and lignin); however, the enzymatic machinery for the depolymerization of cellulose and hemicellulose of cellulolytic fungi was rapidly used and optimized for their utilization in industrial processes, mainly for 2G-bioethanol production. Enzymatic cellulolytic cocktails, derived from fungi such as *Trichoderma*, *Myceliophthora* and others are actually commercially available. However, there is still room for improvement and microbiologists can also help to improve the efficiency in the conversion of raw materials into sugars. Lack of some key activities, especially hemicellulolytic activities in some cocktails, or excessive amounts of unnecessary cellulases have been some of the problems encountered during the evolution of these enzymatic cocktails (Álvarez et al., 2016). The presence of inhibitors and the efficiency of the enzymes when using biomass from different origins and therefore, with different cell wall composition, are also problems that will require optimization of commercial enzymatic cocktails. Utilization of urban waste to biologically generate sugars is another challenge; improvements in the enzymatic cocktails, available for 2G-bioethanol production, are required to efficiently convert this waste into sugars and new processes will have to be designed to couple the production of sugars with SAF production. The production of SAF from lignocellulosic biomass using non-conventional yeasts, such as *Rhodosporidium toruloides* (which can grow using the degradation products of cellulose and hemicellulose and, even, lignin-associated compounds) or microbial consortia or sequential bioprocess with different types of micro-organisms could be an interesting approach to improve the synthesis of SAFs from plant biomass (Walls & Rios-Solis, 2020). Utilization of lignin, one of the main biopolymers on Earth, for the production of fuels, has not yet been extensively explored. As lignin contains aromatic compounds, fuel produced from this raw material could be used without the need to be blended with conventional fuels. It has been demonstrated that the biological depolymerization of lignin could be coupled with the synthesis of valuable compounds (including jet fuel) although huge research efforts are still needed to determine the pathways, improve the productivity and design commercial facilities (Beckham et al., 2016).

In OTJ, triglycerides and fatty acids, principally from vegetal oils (i.e. from *Camelina* or *Jatropha*) but also from used cooking oils, algae or yeasts are being used as starting material (Figure 1). These compounds have to be converted into alkanes through a chemical process. Some of the available SAFs that are currently being commercialized are based on this type of technology. Used cooking oil, for example, is being used by the Chinese company SINOPEC Shenhai to produce HEFA-SAF (hydroprocessed esters and fatty acids-SAF); this company has a 100,000 ton/year facility that has been certified with RSB standards (Roundtable on Sustainable Biomaterials). The use of oleaginous yeasts, such as *Yarrowia lipolytica* or *Aerobasidium pullulans* var *melanogenunm*, capable of accumulating 36% and 66% of CDW as lipids, respectively, represents a very promising option for the synthesis of SAFs. However, as with other microbiological processes, strain improvements and bioprocess optimization are still a pending issue (Lu et al., 2021). Other promising organisms for OTJ are algae, including microalgae. The oil content of some microalgae may exceed up to 80% by weight of its dry biomass. Algae cultivation has additional advantages when compared with plant cultures; they grow at a faster rate and show higher efficiency in photosynthetic activity than crops, therefore, microalgal biomass production has higher yield than plant cultures, they can be cultivated in non-arable land, and the cultivation systems allow the recovery of nutrients from wastewater. Furthermore, the production of SAFs could be coupled with the utilization of residual biomass as organic fertilizers, energy cogeneration or livestock feed, thus improving the economic revenues of the process (Saravanan et al., 2023). Because microalgae are adapted to different environmental conditions, they have a great genetic diversity allowing the design processes to produce not only SAFs but also biogas, bio-oils and bio-hydrogen. They also allow the fast development of improved strains, if required. Despite all the advantages that microalgae present versus oleaginous crops, cultivation scale-up is still a challenge. Light intensity is one of the main limitations in microalgae productivity and it should be taken into account when designing a bioreactor model. Light intensity is variable inside the culture and therefore, reducing culture depth and increasing surface exposure to light should be taken into consideration for modelling of the bioreactor or open pond system. Microalgae are capable of producing 59 m^3 ha^{-1} of algal oil per year, which corresponds to 121 m^3 ha^{-1} $year^{-1}$ of biodiesel (Khan et al., 2018). Replacing all the transport fuel consumed in the United States with biodiesel (approximately 0.53 billion m^3 per year) will require more than 1×10^6 ha, which represents over 1.4 million soccer fields. Therefore, besides improvements in highly efficient photo-bioreactors, reductions in the operational and maintenance costs, as well as in the processes of algae harvest and conversion of oil into final fuels, are still required. Open ponds, which are relatively cheap to construct, are difficult to operate (temperature control or contamination are typical problems in this type of construction), whilst closed bioreactors need energy to operate, supplies of CO_2 (which at large scale could be difficult to provide) and toxic compounds can be formed (Khan et al., 2018). A close collaboration between engineers and scientists is required to improve the technology and to make them profitable. The oil productivity (mass of oil produced per unit volume of the microalgal broth per day) depends not only on the bioreactor design, but also on the algae species used and, therefore, selection of the best organisms that produce high levels of polyunsaturated FAME (fatty acid methyl ester) and have a fast growth rate, can contribute to increase overall productivity. Genetic engineering, to increase the accumulation of fatty acids, lipids or triglycerides and to maximize the efficiency with which solar energy is converted into biomass and bio-products can also increase the viability options, although in many cases the attempts already carried out have not always fulfilled expectations. Improving the cultivation processes, understanding the growth inhibition processes, and nutrient requirements will also increase productivity, whilst reducing the surface of the facility. This is a third generation of fuels, which consists of the use of photosynthetic microbes that utilize CO_2 as the raw material (Saravanan et al., 2023). These autotrophic micro-organisms will allow further increases in the reduction of CO_2 emissions throughout the life cycle of the product. Although currently, jet fuels from HH-SPK or HC-HEFA (hydroprocessed hydrocarbons-synthetic paraffinic kerosene or hydroprocessed esters and fatty acids) are being produced using microalgae, CO_2 fixing bacteria are also being studied for the production of other types of fuels. Synthesis of alka(e)nes and short-chain alcohols, utilization of *Cupriavidus necator* for the production of β-farnesene or engineering yeasts to fix CO_2 are current strategies for fuel production. Bacteria have a faster growth rate and life cycle, and can be genetically engineered with more facility than microalgae and, although there are research efforts involved in discovering new micro-organisms, in engineering and optimizing CO_2 fixation bacterial pathways and enzymes, and engineering yeast for using CO_2, there are very few papers dealing with the microbial production of fuels from CO_2, apart from microalgae utilization (Salehizadeh et al., 2020). The main research lines that have been opened up to now are the conversion of well-known heterotrophs industrial workhorses (such as *Escherichia coli*, or *Saccharomyces*) into autotrophs and the improvement of genetic engineering of autotrophic micro-organisms. Despite all the advances in 3G technologies, most of the approved SAFs use feedstock waste as raw materials (cooking oil, municipal solid waste and others), vegetable oils, or plant biomass (Figure 1).

Renewable hydrogen is one of the most promising fuels for future technologies in aircraft propulsion, yet it is still in infancy. In 2025 Airbus will decide if the market will allow it to support hydrogen-fueled airliners and if it can, the company has planned to use this technology by 2035 (https://www.airbus.com/sites/g/files/jlcbta136/files/2021-07/airbus_hydrogen_future_aviation_1P%20%281%29.pdf). Although biophoto-H_2 production in vivo has been reported in green algae since it began approximately 60 years ago, it has only received a significant attention in the last decade. Biological production of H_2 is still a non-profitable process, mainly due to the low yield and energy conversion efficiency and inhibition of hydrogenase by the oxygen, a by-product of photolysis. New research should be conducted to prevent the inhibition of hydrogenase activity from O_2, to minimize the remainder of competitive electron transport reactions, and to sustainably divert efficient electron flow toward H_2 production, in order to generate the technology necessary to scale-up the process to efficiently produce hydrogen using microalgae (Chen, 2022).

It is, therefore, clear that the production of SAFs using microbes still requires huge research efforts to fulfil the needs of aviation, both in the biological utilization of raw materials as well as in the biological processes to convert these materials (oils, sugars, aromatic compounds and others) into SAFs. However, we should also be aware of the microbiology constraints that, in some cases, will not allow us to reach the commercial level and that, by creating false expectations we will harm the credibility of microbiologists. However, in our opinion microbiologists can and should continue to find new avenues for producing SAFs, and for evaluating the advantages and feasibility of their production. This last step will require a close collaboration between researchers and industry.

AUTHOR CONTRIBUTIONS

Ana Segura: Conceptualization (equal); writing – original draft (lead); writing – review and editing (equal). **Lorena Jiménez:** Writing – review and editing (supporting). **Lázaro Molina:** Conceptualization (equal); writing – original draft (supporting); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals carried out by any of the authors.

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