



D1.1 Overview of biogenic feedstocks

March 2025

Myrsini Christou, Christos Zafiris, Loukas Gavriil, Ioanna Papamichail /CRES Walter Zegada-Lizarazu, Elena Ferro / UNIBO Francisco Gírio, Patrícia Moura, Ana Eusebio, Luis Duarte, Paula Costa / LNEG,



Table 1: Document information

Document Information							
Project name:	ICARUS						
Project title:	International cooperation on sustainable aviation biofuels						
Project number:	101122303						
Start date:	1 October 2023						
Duration:	36 months						

Table 2: Dissemination level of this report

Dissemination level of this report							
PU	Public X						



ACKNOWLEDGMENT & DISCLAIMER

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101122303.

The information and views set out in this report are those of the author(s) and do not necessarily reflect the official opinion of the European Union. Neither the European Union institutions and bodies nor any person acting on their behalf may be held responsible for the use which may be made of the information contained therein.

Reproduction is authorised provided the source is acknowledged.



Executive Summary

SAF is a safe replacement for conventional (fossil-based) fuel that could reduce carbon emissions. It is almost chemically identical to traditional jet fuel. It is generated from feedstocks that absorb carbon dioxide (CO_2) and provide a net reduction in CO_2 emissions when compared to fossil fuels. SAF can be produced from a variety of feedstocks and through several processes. This work analyses and reports the technological developments on the feedstock for SAF production. Focus is set on the sustainable feedstock supply over time. Biomass resources according to RED II Annex IX A (as only advanced biofuels are addressed in the ReFuelEU Aviation) are taken into account. Furthermore, growth of sustainable feedstock supply thanks to improved cropping systems, as well as waste management collection programs, usage of forestry residues and wood processing residues thanks to developments in the gasification technology and new potentials such as SAF production from seaweed and algae is investigated. In addition, this work reviews the status of bioenergy carriers for SAF production like microbial oils (MO), isobutanol and syngas.

Based on the information presented, important feedstocks for SAF production from the agriculture sector are vegetable oils (i.e. rapeseed, carinata, camelina) and non-food cellulosic species (i.e. biomass sorghum, legume cover crops, switchgrass, miscanthus) that can be grown as dedicated energy crops and/or as intermediate crops in several innovative cropping schemes with low ILUC risks and at the same time able to improve soil health and biodiversity. Vegetable oils are suitable feedstocks for SAF through HEFA technologies. On the other hand, lignocellulosic feedstocks are cheaper and with considerably higher biomass yield production potential and can be used as feedstock for the FT synthesis pathways.

Another significant feedstock source for producing SAF is the residues from crops and waste streams. Straw is the most abundant among those sources and the easiest to collect. According to the analysis of Chapter 4, taking into consideration the amount of cereal straw that is not actually exploited for feed production, Europe could technically produce more than 66 million tons of cereal straw per year that can be available for SAF production. Besides straw, a very important source of SAF production feedstock is the lignocellulosic residues from fruit trees, which are basically produced from pruning. Given the current fruit tree area in Europe (11.33 million hectares), around 25 million tons of wood dry weight are produced annually from pruning. Lignocellulosic residues can be produced from forestry as well. Most of the residues coming out from the indirect use of forest wood can be used for biofuel production. According to Eurostat the total available volume of timber is 28,539,588,910 cubic meters, which corresponds to 19,977,712 ktons if we use an average wood density of 700 kg/m³. Dried sewage sludge can be also used as a potential feedstock for the production of SAF through thermochemical pathways. Sewage sludge or residues from industrial processes are wastewaters with high organic content. The energy content of sewage sludge can vary from 19.75 kJ/kgDS to 13.60 kJ/kgDS, Depending on the previous treatment. Total swaged sludge production in Europe for 2021 is estimated to 2,804,940 tonnes of DS.

Finally, municipal solid waste can be a potential source of feedstock for the production of SAF. Carbonbased waste such as product packaging, grass clippings, furniture, clothing, bottles, food scraps and newspapers can be properly processed and converted into SAF. There is an increasing trend in MSW generation, also in recent years an increasing amount of MSW are properly treated and most of the useful fraction of them are recovered, leaving only a small portion for landfilling. These facts justify the importance of this source of potential SAF feedstock. The amount of waste recycled (material recycling and composting) rose from 37 million tonnes (87 kg per capita) in 1995 to 111 million tonnes (248 kg per capita) in 2022 at an average annual rate of 4.0 %. The share of municipal waste recycled overall rose from 19 % to 48 %.



The main conclusion of this work is that there is a lot of untapped potential feedstock that can be used for SAF production, contributing to the effort of reaching the EU targets. But also, there are a lot of technical and logistics issues that must be resolved before this feedstock potential be realistically valorised.

- The innovative multiple cropping systems are still underutilized as SAF feedstock sources. To comply with the increasing demands of SAF feedstocks from the agricultural sector, potential solutions should go, among others, through the development and establishment of long-term innovative low ILUC risk cropping systems.
- To secure large-scale straw supply of satisfactory quality at reasonable prices, straw handling must be carried out as efficiently as possible. Currently, there is a significant amount of expertise in dealing with straw management, and effective logistics and supply chain systems have been established. As a result, delivering straw is not a problem, provided that the required quantities have been secured.
- Collecting wood from pruning presents logistics challenges due to several factors: (i) its dispersion across territories, (ii) the size and layout of plantations, and (iii) the relatively low biomass production per hectare compared to forestry wood. So even if this waste is an excellent biomass source, its utilization is limited due to several constraints such as high costs and lack of technology.
- The process of recovering waste wood from various sources such as municipal waste or different industries is relatively straightforward. The tricky case is to recover wood residues that are left in the forest during the cutting process.
- Vegetable oils present concerns over the low yields of the species that produce them, and therefore high cost and availability.
- Feedstock conversion pathways alternative to HEFA are still complicated and expensive
- Very efficient feedstock supply chains must be established for the resolving problems like:
 - The different harvesting/collection equipment due to high variability of the types of residues (straw, tree pruning, forest residues) with different fuel characteristics
 - The dispersed geographic allocation leads to high transportation costs
 - The location of the feedstock production site and its proximity to conversion plants --> transportation costs
 - The seasonal availability and limited harvesting window requiring time-efficient biomass collection systems
 - The high feedstock prices and limited availability and scalability of sustainable feedstock

Besides feedstock, the energy carriers, an intermediate between raw feedstock and SAF, are very important for the further development of the SAF market. Three key bioenergy carriers were investigated: Non-conventional oleaginous yeasts for optimizing microbial oil, isobutanol production strains, and syngas production.

Oleaginous organisms possess the ability to store a minimum of 20% of their dried biomass as internal lipids. The review of the research TRL on different non-conventional oleaginous yeast species showed that are typically range from TRLs 3 to 4. Currently, two main intracellular processes are recognized for lipid accumulation mechanisms. The "Ex novo" pathway is associated with the presence of hydrophobic feedstock, operating independently of nitrogen depletion. The "De novo" process is more intricate, involving the breakdown of sugars and other hydrophilic substrates. This mechanism is highly dependent on nitrogen scarcity and depletion. Despite their inherent potential, non-conventional oleaginous yeast strains require extensive research and several years of development to attain commercial viability.

Isobutanol has emerged as a promising alternative to ethanol, the most widely produced biofuel globally. Recognizing the limited yields of its production, researchers have implemented various metabolic engineering strategies to enhance the isobutanol biosynthetic pathways in these native host organisms, aiming to boost overall isobutanol synthesis. Ongoing research reflects the dynamic nature



of the field, aiming to overcome limitations and advance the TRL of isobutanol production strains, which is now at Level 3.

Gasification technologies have been used for many years in applications such as heating and power generation. However, its application in drop-in biofuel production using Fischer-Tropsch (FT) synthesis has been based on biomass feedstocks, brings a different set of challenges which have an important impact on the gasification technology selection and on the type of syngas cleaning and conditioning to be carried out. Fischer-Tropsch synthesis process is an already mature technology and, at a theoretical level, does not change with the substitution of fossil feedstocks by biobased ones. Most of the biomass gasification is just approaching commercialization (TRL 7-8), and the jet fuel produced through the FT route has been certified and can be blended up to 50% with fossil kerosene. The first commercial biomass gasification and FT plant, Sierra BioFuels Plant has been constructed by Fulcrum Bioenergy in Nevada, U.S. and started to operate in 2022. Velocys in collaboration with British Airways, is developing first commercial Fischer-Tropsch BtL plant in the UK in Immingham, to produce 75 ML per year Sustainable Aviation Fuel (SAF) from municipal and commercial solid waste.



Table of Contents

Ex	ecutive	Summary	4
Та	ble of C	ontents	7
Lis	st of Fig	ures	9
Lis	st of Tab	ples	10
No	omencla	iture	10
1	Intro	duction	11
2	Scope	e of the work	13
3	Crops	and cropping systems	14
	3.1	Technological status (low TRL (long-term) and high TRL (short-term)	14
	3.1.1	Biomass feedstocks according to RED III Annex IX A	14
	3.1.2	Non-food crops: Characteristics and production potential	15
	3.1.3	TRL and future projections of non-crops grown for SAF production	21
	3.2	Technological innovations	25
	3.3	Technological constraints	29
4	Crop	residues and waste streams	31
	4.1	Technological status (low TRL (long-term) and high TRL (short-term)	31
	4.1.1	Cereal Straw Potential	31
	4.1.2	Potential of other lignocellulosic agricultural residues	35
	4.1.3	Potential of lignocellulosic residues from forestry	39
	4.1.4	Potential of Dried Sewage Sludge	43
	4.1.5	Potential of food waste from MSW	45
	4.2	Technological innovations	50
	4.3	Technological constraints	50
5	Bioer	nergy carriers	51
	5.1	Technological status – low TRL (long-term) and high TRL (short-term)	51
	5.1.1	Non-conventional Oleaginous Yeasts for Microbial Oils (MO) Production:	51
	5.1.2	Strains for Isobutanol Production through Sugar Fermentation:	53
	5.1.3	Syngas production from gasification, gas conditioning and upgrading	56
	5.2	Technological innovations	57
	5.2.1	Non-conventional Oleaginous Yeasts for MO Production:	57
	5.2.2	Strains for Isobutanol Production:	59

🦈 carus

	5.2.3	Syngas production from gasification, gas conditioning and upgrading	60
5	5.3 Т	echnological constraints	60
	5.3.1	Non-conventional Oleaginous Yeasts for MO Production:	60
	5.3.2	Strains for Isobutanol Production:	61
	5.3.3	Syngas production from gasification, gas conditioning and upgrading	62
6	Conclu	isions	63
7	Refere	nces	65



List of Figures

Figure 1-1: Historical and predicted CO ₂ emissions from the aviation industry from 1990 to 2050 11
Figure 3-1: Main SAF feedstocks and their level of development
Figure 3-2: Multicropping systems concept (source: FAO, 2012)
Figure 3-3: Effects of intercropping and N fertilisation on biomass production of grass and legume crops in two consecutive growing seasons. Different lowercase letters indicate significant differences between crops and cropping systems. Different uppercase letters indicate significant differences between N fertilisation levels. PM, pearl millet; S biomass sorghum; SH, sunn hemp. (Zegada-Lizarazu et al. (2021a)
Figure 3-4: Biomass energy [Ln(GJ ha-1) generated per cropping system. Numbers at the top and inside the bars are the back-transformed means (GJ ha-1). Different lowercase letters indicate significant differences among treatments. (Zegada-Lizarazu et al. 2023)
Figure 4-1: Total cereal straw production in EU (in tons) in descending order
Figure 4-2: Fruit tree area in the EU (Aliano-González et al. 2022)
Figure 4-3: Distribution of fruit tree areas in EU countries (Aliano-González et al. 2022)
Figure 4-4: Distribution of olive trees and grapevines (Aliano-González et al. 2022)
Figure 4-5: Nut trees, apple and pear trees, stone fruit trees, citrus fruit trees, and other trees (Aliaño- González et al. 2022)
Figure 4-6: Classification of the wood-based fuels according to CEN/TS 14961. Source: VTT
Figure 4-7: Primary supply of solid biomass in 1000 m3 for energy production, indigenous production in 2021 grouped by feedstock origin (EC, 2024)
Figure 4-8: Logging residues valorisation (VTT)
Figure 4-9: Municipal solid waste generated in Europe in 2004 and 2022
Figure 4-10: Municipal solid waste generated treatment in Europe 1995-2022



List of Tables

Table 1: Document information
Table 3-1: Summary of major changes to transport fuels policy in the Renewable Energy Directive 14
Table 3-2: Requirements for minimum volume shares of SAF and synthetic aviation 15
Table 3-3: Fatty acid percentage of the main vegetable oils. 16
Table 3-4: Lignocellulosic content of main dedicated energy crops. 19
Table 3-5: Feedstock availability and TRL level for the different SAF pathways (Shehab et al. 2023) . 23
Table 3-6: Expected TRL level by 2030 of relevant advanced biofuel agricultural feedstocks. + TRL 3-5; ++ TRL 5-7; +++ TRL >7. Agricultural practices: intercropping (I), cover cropping (CC), rotation (R), agroforestry (AF), biochar (B). (Panoutsou et al. 2022)
Table 3-7: Mineral concentration in several intercropping systems. (Zegada-Lizarazu et al. 2021a) 27
Table 3-8: Biomass and seed yield, and energy balance of different relay cropping systems at threelocations in United States (modified from Berti et al. 2015)28
Table 4-1: Available straw for SAF production in EU Countries (Eurostat 2022)
Table 4-2: Lignocellulosic feedstock characteristics
Table 4-3: Fuel characteristics of feedstock from trees. 38
Table 4-4: Tree pruning fuel characteristics
Table 4-5: Wood area of EU-27, ha
Table 4-6: Volume of timber for the EU-27 countries, thousand m ³
Table 4-7: Forestry wood feedstock characteristics
Table 4-8: Sewage sludge production from WWTP in thousands of tons (EUROSTAT 2024)
Table 4-9: Calorific Values from waste water treatment plants – Low heating value
Table 4-10: Amount of municipal waste treated in the EU for the period 1995 to 2022
Table 4-11: Food waste generation for 2020 (EUROSTAT) 49
Table 5-1: Review on TRL for non-conventional yeasts 52
Table 5-2: Summary of isobutanol producing hosts. 54

Nomenclature

MSW: Municipal Solid Wastes SAF: Sustainable Aviation Fuels TRL: Technology Readiness Level WWTP: Waste Water Treatment Plants



1 Introduction

Efficiency has always been a tremendous driver of progress in aviation and has made air travel and mobility central to modern life. Aviation's drive for the reduction of fuel utilization and higher operational efficiency has helped the industry limit its emissions. In 2021, the aviation industry established a challenging goal to achieve net-zero carbon emissions by 2050. Sustainable aviation fuel (SAF) has a crucial role to play in providing a cleaner source of energy to power the world's fleet of aircraft and help the billions of people who travel by air each year to lower the impact of their journeys. The industry's Waypoint 2050 analysis suggests that SAF will contribute between 53 and 71% of the emissions reductions needed to get to net-zero by 2050. There were 46.8 million scheduled commercial flights carrying 4.5 billion passengers in 2019, which generated roughly 2% of global human induced carbon emissions equivalent to 914 million tonnes of CO₂. Aviation's passenger numbers are expected to grow to up to 7.2 billion by 2035, while the global annual SAF production is estimated to reach 26 million tons (32.5 billion litres) by 2050 (Wang et.al, 2024), meaning that effective action on reducing carbon emissions is essential to ensure the sustainable development of the industry.

Companies across this sector are collaborating to reduce emissions through a four-pillar strategy of i) new technology; ii) operations and infrastructure improvements; iii) SAF adoption; and iv) out-of-sector market mechanisms to fill the remaining emissions gap.

Using SAF provides the aviation industry with an alternative energy source, enables the industry to reduce its carbon footprint by reducing its dependence on fossil-based fuel sources and allows it to draw upon a variety of different energy providers. SAF will play a key role in achieving the industry's goal of net-zero carbon emissions by 2050. Figure 1-1 illustrates the historical and predicted CO_2 emissions from the aviation industry from 1990 to 2050, taking into account planned mitigation measures.



Figure 1-1: Historical and predicted CO₂ emissions from the aviation industry from 1990 to 2050

SAF is a safe replacement for conventional (fossil-based) fuel that could reduce carbon emissions. It is almost chemically identical to traditional jet fuel. It is generated from feedstocks that absorb carbon dioxide (CO_2) and provide a net reduction in CO_2 emissions when compared to fossil fuels. Today, SAF is blended with conventional kerosene in ratios of up to 50% SAF to ensure compatibility with aircraft, engines, or fuelling systems. Commercial flights are currently permitted to fly with a blend of SAF and conventional fossil-based kerosene. The industry is working towards commercial aircraft being permitted to fly with 100% SAF shortly.



SAF can be produced from a variety of feedstocks and through several processes. Importantly, the aviation industry has committed to ensuring that sustainability is the highest priority for the development of this new energy source.

It is an alternative to traditional energy sources for aviation, in this case non-conventional or advanced fuels, and includes any materials or substances that can be used as fuels, other than conventional, fossil sources (such as oil, coal, and natural gas). It is also processed to create jet fuel in an alternative manner. Feedstocks for SAF are varied; ranging from cooking oil, plant oils, municipal waste, waste gases, agricultural residues, green hydrogen and even electricity – to name a few. It is important to note that not all "alternative" fuels are "sustainable".

Aviation fuel refers to drop-in fuel that meets the technical requirements for use in commercial aircraft and can be used in existing technology and fuel systems, ensuring the most important aspect of aviation operations – safety – is maintained.

Current technology allows sustainable aviation fuel to be produced from a wide range of feedstocks, including:

- Residual streams:
 - Waste oils and fats: this typically comes from plant or animal fats and greases that have been used for cooking and are no longer usable for further cooking (used cooking oil), or as waste from food production (such as tallow).
 - Municipal solid waste: carbon-based waste that comes from households and businesses. Some examples include: product packaging, grass clippings, furniture, clothing, bottles, food scraps and newspapers.
 - Cellulosic waste: this comes from excess wood, agricultural waste (such as corn stalks), and forestry residues (branches and leaves that are not tradeable).
- > Crops
 - Cover crops such as camelina, carinata, and pennycress: cover or rotational oil seed crops that are grown in rotation with wheat and other cereal crops within the same year, when the land would otherwise be left fallow (unplanted) as part of the normal crop rotation programme.
 - Jatropha: a plant that produces seeds containing inedible lipid oil that can be used to produce fuel. Each seed produces 30 to 40% of its mass in oil.
 - Halophytes: salt marsh grasses and other saline habitat species that can grow either in salt water or in areas affected by sea spray where plants would not normally be able to grow.
- Algae
 - Algae: these are microscopic plants that can be grown in plastic sleeves (micro algae) or polluted or salt water, deserts and other inhospitable places (macro algae).
- Non-biogenic alternative fuels: these include 'power-to-liquid', which typically involves creating SAF from carbon sources such as industrial point source waste gases or, in the future, direct air captured carbon, combined with green hydrogen produced using renewable energy powered electrolysers. Alternatively, industrial waste gases can be converted into ethanol using biological conversion processes, and the ethanol subsequently converted into jet fuel.

RED II Annex IX A determines the sustainability of all the above feedstocks.



2 Scope of the work

This work aims to analyse and report the technological developments on the feedstock for biojet production. Focus will be set on the sustainable feedstock supply over time. Biomass resources according to RED II Annex IX A (as only advanced biofuels are addressed in the ReFuelEU Aviation) will be taken into account.

Furthermore, growth of sustainable feedstock supply thanks to improved cropping systems, as well as waste management collection programs, usage of forestry residues and wood processing residues thanks to developments in the gasification technology and new potentials such as biojet production from seaweed and algae will be investigated. CRES and UNIBO collected information of the crop feedstocks and NEVIS on waste streams management.

In addition, this work aims to review the status of bioenergy carriers for SAF production (BIOREF, LNEG):

- i) non-conventional oleaginous yeasts available to produce microbial oils (MO) and best environmental conditions that would enhance MO yields,
- ii) most promising strains for isobutanol production through sugar fermentation and environmental conditions that would enhance isobutanol yields and
- iii) approaches for syngas production from gasification, gas conditioning and upgrading.

This deliverable will report the finding that will serve as inputs for WP2 'Innovations in SAF technologies in Europe.



3 Crops and cropping systems

3.1 Technological status (low TRL (long-term) and high TRL (short-term)

3.1.1 Biomass feedstocks according to RED III Annex IX A

Sustainable biofuels are important to increase the share of renewable energy in sectors that are expected to rely on liquid fuels in the long term. According to RED III Annex IX, aviation fuels mean advanced biofuels produced from the feedstock listed in Annex IX, part A of the Directive. These materials encompass a variety of sources such as agricultural crop residues, forest residues, dedicated energy crops, organic waste and algae. The directive delineates specific criteria and sustainability standards for sourcing and utilizing biomass feedstock to ensure environmental responsibility and mitigate adverse effects on biodiversity and land use.

An important source of advanced biofuels produced from agriculture is non-food cellulosic materials. According to article 2 (42) of the Directive it refers to feedstock mainly composed of cellulose and hemicellulose, with lower lignin content, including food and feed crop residues, grassy energy crops, and intermediate crops (as catch crops and cover crops, provided that the use of such intermediate crops does not trigger demand for additional land). Such intermediate crops could be produced in several innovative cropping schemes with low ILUC risks, increased sustainability, and enhanced ecosystem services (i.e. intercropping, double cropping, relay cropping). Currently there is no explicit ban on intermediate crops, as provided by the ReFuel Aviation regulation.

	2018 RED II	2023 RED III			
Renewable energy in transport	14% energy target (out of road and rail fuels)	14.5% GHG intensity reduction target or 29% renewable energy target (out of all energy supplied to transport)			
Advanced biofuels (Annex IX, part A)	3.5% (out of road and rail fuels, with multiplier)	5.5% of a combination of both fuel types, with a 1% RFNBO			
Renewable fuels of nonbiological origin (RFNBOs)	No target	minimum (out of all energy supplied to transport)			
Waste oils (Annex IX, part B)	1.7% cap (out of all energy supplied to road and rail)	1.7% cap (out of all energy supplied to transport)			
Food- and feed-based biofuels	Cap at whichever is lower: 7% or 2020 consumption in each member state + 1% (out of road and rail fuels)	Cap at whichever is lower: 7% or 2020 consumption in each member state + 1% (out of all transport energy consumption)			
Multipliers	 2x for advanced biofuels and waste oils 4x for renewable electricity used in vehicles 1.5x for renewable electricity in rail 1.2x for aviation and maritime fuels, except food- and feedbased biofuels 	 Towards the overall 29% renewable energy target and all applicable subtargets for either an energy target or GHG target: 2x for advanced biofuels, RFNBOS, and waste oils 4x for renewable electricity in vehicles 1.5x for renewable electricity in rail 1.2x for advanced biofuels and 1.5x for RFNBOS in aviation and maritime sectors 			
Fossil comparator	 94 gCO₂e/MJ for all transport energy 	 183 g CO₂e/MJ for electricity used in vehicles 94 g CO₂e/MJ for all other energy used in transport 			

Table 3-1: Summary of major changes to transport fuels policy in the Renewable Energy Directive

The ReFuelEU Aviation regulation will establish EU-wide blending targets for renewable fuels in aviation, commonly referred to as sustainable aviation fuels (SAF), from 2025 to 2050 according to the schedule shown in Table 3-2. Such timetable suggests that the demand of feedstock from sustainable sources will significantly increase in the near future. Fewer types of biofuel feedstock may be utilized in the production of SAF compared to RED III Annex IX, part A. However, all biofuels meeting the sustainability criteria set up in the RED III can be used for SAF production. Moreover, feedstocks listed in annex IX, part B can contribute towards fulfilling the SAF targets.



Effective dates	Sustainable aviation fuel	Synthetic aviation fuels
January 1, 2025	2%	
January 1, 2030	6%	
January 1, 2030 - December 31, 2031		Average share of 1.2%, with a minimum share of 0.7% in each year
January 1, 2032 – to December 31, 2034		Average share of 2%, with minimum shares each year of 1.2% from January 1, 2032, until December 31, 2033, and 2% from January 1, 2034, to December 31, 2034
January 1, 2035	20%	5%
January 1, 2040	34%	10%
January 1, 2045	42%	15%
January 1, 2050	70%	35%

Table 3-2: Requirements for minimum volume shares of SAF and synthetic aviation

3.1.2 Non-food crops: Characteristics and production potential

This report will focus only on non-food crops, which will allow the scaling up of SAF production without undermining food security and aligning with broader sustainability development goals and climate change mitigation.

Non-food crops, in particular dedicated perennial grasses or oil crops can be grown on marginal lands that are unsuitable for food crops, thereby reducing the pressure on arable land. Moreover, non-food crops can be grown in sustainable cropping systems that lead to increased biomass production, while at the same time improving soil health and biodiversity. Such practices can help build supply chains for SAF production less affected by food market fluctuations, provide economic opportunities for farmers and support rural economies.

Further to the advantages above, the cultivation of non-food crops can initiate technological and research opportunities for innovations on crop development and advances in the processing technologies. Investing in research for non-food crops can lead to the development of high-yield, resilient plant varieties specifically optimized for fuel production, whereas research into the conversion processes for non-food crops can lead to more efficient and cost-effective methods for producing SAF.

3.1.2.1 Oilseed crops

Non-edible vegetable oils from various plant species are promising sources of feedstocks to produce aviation fuels. Most of these species have the versatility to be grown in rotation as an annual crop or as cover (intermediate) crops in agricultural but also on marginal soils with low fertilization and pesticide inputs. Some members of the Brassicaceae family for example are considered more tolerant to extreme weather conditions than other oilseed crops therefore an attractive option for SAF production in marginal conditions (Kaltschmitt and Neuling 2017). Below are reported the most widely grown non-food oilseed crops and some new promising species as SAF feedstocks in Europe and globally.

Oil crops characterisation is depicted in Table 3-3 (Gopinathan and Sudhakaran 2009):



Crop	Palmitic	Palmitoleic	Margaric	Stearic	Oleic	Ricinoleic	Linoleic	Linolenic	Arachidic	Erucic	
Castor	1.36	-	-	1.11	3.37	88.07	4.82	0.56	0.25	-	
Jatropha	14.39	0.69	0.08	5.83	42.05	-	35.90	0.23	0.09	-	
Palm	42.31	0.17	0.06	4.27	40.90	-	10.07	0.28	0.31	-	
Rapeseed	4.06	0.23	0.07	1.54	62.29	-	29.65	8.71	0.87	0.77	
Soybean	11.50	0.16	0.04	4.11	23.50	-	53.33	6.76	0.32	0.07	
Sunflower	6.35	0.07	-	3.92	20.91	-	67.58	0.17	0.22	-	
Peanut	10.33	-	-	2.79	49.63	-	31.52	0.64	1.07	0.10	

Table 3-3: Fatty acid percentage of the main vegetable oils.

Non-food oilseed crops suitable to EU environments

1) <u>Camelina</u>

Camelina (Camelina sativa), a new crop to EU environments, is an annual oilseed species belonging to the Brassicaceae family, called false flax, linseed dodder, or gold-of-pleasure. Camelina's adaptation to vast areas of the world, combined with its unique oil composition and properties useful for the production of biofuels, jet fuel, biobased-products, feed, and food has renewed interest in this ancient crop (Berti et al. 2015). In EU the cultivation of camelina is at its early stages, it is estimated that about 10000 ha are cultivated with camelina (Zanetti et al. 2021), however official data on the exact surface area dedicated to this species is not available. Both spring and winter annual biotypes of camelina are available, but the possibility of sowing spring camelina in autumn is confined to regions where the winter air temperature never exceeds – 10 to – 14 °C (Soorni et al. 2017). Its growth cycle ranges between 90 and 250 days (Czarnik et al. 2018). Camelina seed production in European environments ranges between 1.3 and 3.3 Mg DM ha⁻¹ (Angelini et al. 1997). Seed yield is strongly affected by weather conditions and is higher with milder temperatures during the growing season (Jankowski et al. 2019). Moreover, increasing sowing density and N fertilization results in greater production (Czarnik et al. 2018). Seed oil content has been reported to range from 300 to 490 g kg-1 (Vollmann et al. 1996; 2007). Camelina oil is rich in oleic, (18:1, 14–16%), linoleic (LA), (18:2, 15–23%), alfa-linolenic (ALA), (18:3, 31–40%), and eicosenoic (20:1, 12–15%) acid. Other minor fatty acids include palmitic (16:0), stearic (18:0), and erucic (22:1) acid (Putnam et al. 1993). Sustainable aviation fuel derived from camelina is produced through hydro-processing and is commonly known as HRJ or hydro-processed esters and fatty acid (HEFA) fuels.

2) <u>Carinata</u>

Brassica carinata, commonly referred to as "Ethiopian mustard," "Ethiopian rape," "Abyssinian mustard," or "carinata," is being developed as a low carbon intensity, non-food oilseed feedstock to produce advanced drop-in renewable fuels, protein-rich meal, and bio-products (Seepaul et al. 2020a, 2020b, 2021, 2023). Carinata is also member of the family Brassicaceae considered a promising new species in EU currently being largely investigated. The origin of the oilseed crop makes it well adapted to its native habitat, the highlands of Ethiopia, in cold temperatures of 14–18°C at elevations of 2200–2800 m above sea level (Persaud et al. 2022). It is heat tolerant, resistant to diseases and seed shattering. It has lower water requirements than other oilseed species of the same family (Kumar et al. 1984). Carinata is tolerant to a wide range of climatic conditions and can be fall-planted in the humid subtropical regions with mild winters or spring-planted in the humid continental climate with hot and humid summers like those of EU. Carinata seeds has 18.7%–28.3% protein and 42%–52% oil content



with a well-distributed fatty acid profile. Erucic acid (41%–43%) forms the primary fatty acid component, followed by linoleic, linolenic, and oleic acids (Kumar et al. 2020). Oil concentration responds to nutrient management, particularly N application rates. An increase in N application rates resulted in a decrease in oil content, and an increase in protein content as oil and protein concentrations are inversely related (Hossain et al. 2018). The oil obtained from the seeds of carinata could be refined to produce SAF due to high concentration of erucic acid in the seed. The SAF derived from carinata could help the aviation sector mitigate CO_2 emissions.

3) <u>Castor</u>

Despite its high suitability to European climates, castor crop is inexistent in the European farming systems. Castor (*Ricinus communis*) is a member of the Euphorbiaceae family; this plant originates in Africa but is found in both wild and cultivated states in all the tropical and subtropical countries of the world (Forero 2005). It is originally a tropical season perennial plant that can also grow in temperate climates as an annual crop (Barnes et al. 2009). Castor bean has been used for years as an industrial oilseed crop because of its high seed oil content of approximately 46–55 wt % (more than twice as much as soybean), its unique fatty acid composition and lubricity (i.e. hydroxy fatty acid ricinoleate), and its ability to grow under varying weather conditions (Table 3-3). The oil yield from castor can be as high as 2 Mg ha⁻¹. Castor oil is obtained from castor seeds, which are poisonous to humans and animals due to the presence of ricin, ricinine, and other allergens, which are toxic (Ogunniyi 2006). Meal from the oil extraction is toxic and cannot be used as fodder without detoxification (Barnes et al. 2009). Several experimental studies on the hydroprocessing of castor oil indicate its suitability to produce jet fuel (Molefe et al. 2019; Liu et al. 2015).

4) Pennycress

Field pennycress (Thlaspi arvense) or simply pennycress is a winter annual weed classified as a member of the Brassicaceae family (Alhotan et al. 2017). It is characterized by low temperature tolerance, a short life cycle, and is adapted to being farmed with existing equipment. Appears to be an ideal candidate to replace winter fallow between typical summer cash crops, like corn, soybean or sunflower (Sindelar et al. 2017). Pennycress, which is native of Eurasia, possesses many traits that can easily support its integration into existing European and Northern American crop rotation systems (Jordan et al. 2016). However, pennycress is not cultivated in Europe and, in contrast to the USA, the research and available information is limited. It can be grown as a winter cover crop while also supplying farm producers with direct economic benefits. As a winter cover in double crop rotations, pennycress is established in the fall and harvested in mid-to-late-spring (Markel et al., 2018). The seed yields in North Dakota are of 1.5 Mg ha⁻¹ (Carr 1993). In Illinois is reported that wild type strains planted in prepared ground resulted in seed yields of 900 kg ha-1 to over 2352 kg ha-1. Commercial strains with genetically improved research lines are now exceeding 2463 kg ha-1 (Phippen et al. 2010) indicating that higher yields are possible. Pennycress has a high oil content (\sim 36%) in the seeds (Alhotan et al. 2017) and is considered a typical non-food oilseed crop (Sedbrook et al., 2014) because of its high erucic acid content (> 300 g kg-1 of its total seed oil DM) and seed meal that contains glucosinolates, making any food/feed use of the seeds less desirable. Within the last decade, pennycress oil, in view of the high content of monounsaturated fatty acids (MUFA) (Moser et al. 2009), has attracted increasing interest as raw material for biodiesel and/or jet fuel production in the USA (Sindelar et al. 2017). It has the potential to be used as feedstock for biofuels with a low impact on the food supply or land use (Zanetti et al. 2019). The oil extraction step for pennycress yields a meal that is rich in protein content (31%) (Fan et al. 2013). However, the meal contains high levels of glycosinolates and erucic acid, which might prevent its use as fodder (Alhotan et al. 2017).



5) Solaris energy tobacco

Tobacco (Nicotiana tabacum L. cv. Solaris) is a widely cultivated plant throughout the world, with its leaves used for the production of smoking products. Tobacco seeds are considered a by-product of the tobacco leaf production, and they are mostly left in the field unused (Barla et al. 2019). These seeds have a moderate oil yield, which can be used for biofuel production (Poltronieri 2016). Tobacco is indeed an oilseed crop with an oil yield ranging from 30 to 40 % of seed dry weight (Grisan et al. 2016). A nicotine-free version of the tobacco plant non-genetically modified organism was developed to be used as biomass feedstock. Energy tobacco (also known as Solaris), unlike the tobacco used for smoking, contains no nicotine in the leaves and maximizes the production of flowers/seeds, reducing leaf growth (Grisan et al. 2016). It is cultivable in Europe, which has strong tobacco cultivation experience, while other second-generation crops are not. In Central Italy seed production was evaluated to be 1.1–1.8 Mg ha⁻¹, with an oil yield up to 0.59 Mg ha⁻¹. In Northern Italy, two seed harvests determined a total seed yield of 4.5 Mg ha⁻¹, from which 1.48 Mg ha⁻¹ of oil could be obtained. The cultivar Solaris was extremely adaptable in terms of morphological parameters and seed yield to different management practices as well as climatic conditions (Grisan et al. 2016). The meal from the oil extraction step can be used as animal feed with its high crude protein content (33%) (Rossi et al. 2013).

6) <u>Salicornia</u>

Salicornia (*Salicornia bigelovii*) is a leafless, annual salt marsh plant native to North America and the Caribbean (Lonard et al. 2012) with green, succulent, jointed stems that terminate in fruiting spikes on the upper one-third of the plant with many small (0.6 to 0.9 mg) oilseeds (Glenn et al. 1991). Salicornia is a member of the Halophyte family, which is known for its ability to grow in brackish water on marginal lands (Sharma et al. 2016). They can be irrigated with seawater without compromising their biomass and seed yields making them good alternative as bioenergy crops. Both oils produced from the seeds and the lignocellulosic biomass of halophytes can be utilized for biofuel production (Sharma et al. 2016). Salicornia produces about 2 Mg ha⁻¹ seeds per hectare and can store oils up to 30% of the total dry weight (Glenn et al. 1999). Studies show that the fatty acid methyl ester composition of oils extracted from halophytes is comparable to the other oil crops used for production of biodiesel (Abideen et al. 2012; Gul et al. 2013). Moreover, Salicornia straw can be gasified and converted into other energy products via Fischer Tropsch (FT) synthesis, pyrolysis, etc. (Warshay et al. 2017)

7) <u>Jatropha</u>

Jatropha (*Jatropha curcas*) is a native of Mexico and tropical South America, but is now naturalized in many tropical and subtropical countries of the world. It is commonly known as physic nut. This crop is drought tolerant, pest resistant, have a rapid growth, easy propagation, higher oil content than other oil crops (40-60%), small gestation period, and adaptation to a wide range of environmental conditions (Singh et al. 2013). A seed yield of 4–5 Mg ha⁻¹ would be reasonable for its commercial viability (Table 3-3; Gopinathan and Sudhakaran 2009). Jatropha-based HEFA fuel was one of the first SAFs to be used for flight tests. The prospect of high oil yield, along with the capability of the plant to grow on marginal lands with low input, made jatropha appealing as a biodiesel feedstock in the early 2000s (Singh et al. 2012). However, Jatropha has lost interest because the real yields at large scale plantations are far lower than previously predicted. Moreover, other constrains such as limited seed availability, high plantation and harvesting costs due to continuous fruiting hampers the cost effectiveness of biodiesel production from such feedstock. Oil extraction step from jatropha produces husk and shell in addition to the meal. The meal from Jatropha is toxic and cannot be utilized as fodder unless it is detoxified (Makkar 2016).



3.1.2.2 Lignocellulosic crops

Lignocellulosic materials are non-edible biomass, making them particularly attractive as feedstock for biofuel production due to their relatively low cost, abundant availability and sustainability. This biomass, not suitable for consumption, avoids competition issues with food crops. However, it necessitates a conversion process with numerous steps due to its high oxygen content, impacting in the fuel costs and reduction technology readiness level as mentioned before.

Lignocellulosic feedstock can be divided into several groups, including agricultural residues, forest biomass and residues, dedicated energy crops (perennial grasses and other dedicated energy crops; Table 3-4), as well as industrial and municipal solid waste (MSWs) (Pasa et al. 2022).

Dedicated energy crops represent promising sources of lignocellulosic biomass due to their low demand for fertilizers and pesticides, as well as their minimal requirement for agricultural land. Furthermore, energy crops grown in warm and temperate regions are among the world's highest-yielding biomasses, producing more than twice the amount with high cellulose content compared to other types of crops.

Lignocellulosic crops suitable to EU environments

1) <u>Sorghum</u>

Due to its large plasticity, sorghum is increasingly being used as animal feed, fodder, and biomass crop worldwide. Its genetic diversity provides a large range of stem biochemical composition suitable for various end-uses such as lingocellulosic, sugar and /or starch non-food feedstock that can be grown as intermediate crop within conventional crop rotations. Its drought, waterlogging and salinity tolerance renders it a highly productive crop in environmental conditions where the cultivation of other cereals is restricted. Chilling stress, however, is a limiting factor to take into consideration. Sweet/biomass sorghum specifically accumulates substantial quantities of soluble sugars (sucrose, glucose and fructose) and structural sugars (cellulose, hemicellulose, and lignin) in its stems. Whereas the starch in the seeds reach about 75 %. It can be used for advanced biofuels production as well as for alcohol to jet routes (Zegada-Lizarazu and Monti, 2012). In the EU, production of sweet/biomass sorghum for advanced biofuels is almost inexistent, though it can be cultivated throughout most of Europe with one of the highest biomass yield rates among lingocellulosic feedstocks.

Energy crops	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Switchgrass	45	31	12
Miscanthus	38-40	18-24	24-25
Giant reed	30-32	32-38	18-19
Elephant grass	22	24	24
Grass Esparto	33-38	27-32	17-19
Napier Grass	32	20	9

Table 3-4: Lignocellulosic content of main dedicated energy crops.



2) <u>Switchgrass</u>

Switchgrass (*Panicum virgatum* L.) is a perennial species, native to North America, thrives in marginally productive croplands. It holds significant potential as a bioenergy feedstock for producing cellulosic ethanol, as well as for heat and electricity generation through direct combustion, gasification and pyrolysis. Switchgrass consistently achieves high yields compared to other species across diverse environments, while also demanding minimal agricultural inputs. Beyond its role in bioenergy production, switchgrass contributes to soil and water conservation, carbon sequestration and wildlife habitat enhancement. In the first year after establishment, switchgrass commonly achieves 75-100% of their potential yield, amounting to 8-13 Mg ha⁻¹ in dry matter basis, with potential ethanol yields estimated between 3740 and 5620 L ha⁻¹. According to Roozeboom et al. (2019), in an 11- year long-term production system, switchgrass yielded 11.3 Mg ha⁻¹ of dry biomass and an estimated total ethanol yield of 3.8 m³ ha⁻¹. Moreover, switchgrass-based ethanol production resulted in greenhouse has emission that were 94% lower compared to estimated emissions from gasoline (Umakanth et al. 2022).

3) Miscanthus

Giant miscanthus (*Miscanthus x giganteus Greef* et Deu.) is a perennial, warm-season grass native to Asia, characterized by the C4 photosynthetic pathway. It exhibits cold tolerance and thrives in cool temperatures, yielding high biomass. Additionally, it shows resilience on marginal lands and withstands certain levels of flooding. Extensively researched in the European Union, where its production is primarily concentrated, giant miscanthus is commercially utilized for bedding, heat and electricity generation. In the United States, it serves as a prominent feedstock for cellulosic ethanol, being more conductive to thermochemical conversion into biofuel than biochemical methods. Distinguished by its high yields, especially in cool temperatures, miscanthus can exceed those typical yields of switchgrass by more than double. Harvestable yields range from 10 to 30 Mg DM ha⁻¹ depending on location and inter annual weather variations during the growing season. The ranges for hemicellulose, cellulose and lignin are 295-303 g kg⁻¹, 446-458 g kg⁻¹, and 70-80 g kg⁻¹, respectively. In terms of ethanol production, miscanthus exhibited greater total ethanol production compared to switchgrass (5594 vs 3699 L ha⁻¹) (Umakanth et al. 2022).

4) Reed canary grass

Reed canary grass (*Phalaris arundicea*), a member of the Poaceae family, is a native perennial grass found in temperate regions across Europe, Asia and North America. Particularly abundant in Nordic countries in Europe, it thrives in wet environments such as lakeshores. In North America, it is primarily utilized as a forage crop, although its primary purpose has shifted towards energy production. It is well suited for cultivation in poorly drained soils due to its tolerance to flooding and it also exhibits good resistance to drought (Stefanoni et al. 2023). Canary grass yields reach 8-10 t ha⁻¹ (Wang et al. 2023). The relatively low levels of lignin (19.4% DM) compared to cellulose or hemicellulose in reed canary grass, are indicative of the potential of the species as a fermentation feedstock for bioethanol. Moreover, reed canary grass shows superior bioethanol conversion potential compared to switchgrass after pre-treatment and ensiling, with overwintered spring harvest material apparently more amenable to hydrolysis than that cut in the fall.

5) <u>Giant reed</u>

Giant reed (*Arundo donax* L.) belongs to the Poaceae family, is an herbaceous perennial crop known for its ability to thrive in different environmental and soil conditions. Resistant to numerous pests and disease, it offers relatively high biomass yields requiring minimal agronomic inputs. Primarily cultivated for grassland management, phytoremediation and bioenergy production, Arundo boasts several advantages over other energy crops. These include its adaptability to varying environmental and soil conditions. Additionally, research has shown its capability to yield substantial biomass even in high-salinity environments. Moreover, it is well-suited for cultivation on marginal or sub marginal lands, including polluted areas and infertile soils (Stefanoni et al.



2023). Giant reed can produce promising yields of about 25 t ha⁻¹ per year. Giant reed can be considered good feedstock for advanced biofuels production, thanks to its high calorific value (18.27 MJ kg⁻¹) and hemicellulose contents (31% cellulose and 35 % hemicellulose). Some studies indicate that giant reed can produce 50% more ethanol than sugarcane or sugar beet, 20% more than miscanthus and about three times more ethanol per hectare than maize.

3.1.3 TRL and future projections of non-crops grown for SAF production

Vegetable oils derived mostly from seeds of certain crop species are the main feedstock to produce SAF through the HEFA pathway. In fact, HEFA fuels and its feedstocks are about to enter the market thanks to their high TRL (Figure 1-1) and low processing costs.

Many feedstocks suitable for HEFA conversion are already used in the road sector and could be diverted to aviation, depending on availability, production schemes, policy incentives, etc. (O'Malley et al. 2023). It is expected for example that soybean-based HEFA will provide substantial volumes of SAF in the next decade, especially in USA and Brazil where soybean feedstock production systems are well grounded and commercially available (Figure 3-1). While in Indonesia biofuel manufacturers primarily use palm oil to produce biodiesel and the EU, is focusing on rapeseed oil.

Other oilseed crops, with relatively lower TRL level in agronomic terms, are being tested worldwide including ricinous (*Ricinus communis*), camelina (*Camelina sativa*), pennycress (*Thlaspi arvense*), tallow tree (*Triadica sebifera*), and carinata (*Brassica carinata*) among others. Mofijur et al. (2023) indicated that *Ricinus communis* is the highest quality-ranked feedstock for sustainable aviation fuel production based on its fatty acid composition and properties (i.e. kinematic viscosity, density, cetane number, higher heating value, iodine value, oxidation stability, and cold filter plugging point) followed by Neem (*Azadirachta indica*), and pequi (*Caryocar brasiliense*). Nevertheless, all these oilseed crop species are considered high-risk feedstocks, constrained mostly by their limited availability, low intrinsic yield potential, and their geopolitical and geographical limitations (O'Malley et al. 2023; Shehab et al. 2023).



Figure 3-1: Main SAF feedstocks and their level of development

At present, it is foreseen that in 2024 HEFA production from soybean oil, rapeseed and palm oil will flatten, while second generation cellulosic SAF derived from dedicated lignocellulosic crops, perennial grasses and/or lignocellulosic agricultural residues will continue to grow and become the preponderant feedstocks for SAF production by the end of the decade (O'Malley et al. 2023). These feedstocks are considered more suitable for FT-gasification production pathway. However, plant-based lignocellulosic feedstocks require significant processing in order to extract the basic sugar components that can be converted into SAF.



Various companies around the world are developing different types of technologies to process cellulosic feedstocks, however these technologies have not yet been demonstrated at commercial scale. Moreover, even though lignocellulosic feedstocks are abundant and have the potential to scale up, the agronomic management of these lignocellulosic species is at variable TRL levels (Table 3-6) depending on the traditional and/or new end uses of such species. By 2030 though it is expected to see a significant increase in their agronomic and mechanization management so to reach a high TRL level (Table 3-6). For example, most of the dedicated lignocellulosic crops (i.e. switchgrass, miscanthus, giant reed) are largely undomesticated and are at their early stages of development and improvement. Lignocellulosic agricultural residues (i.e. maize stover, wheat straw) come from well-developed and largely commercialized species. However, whatever the lignocellulose sources, their contribution to SAF production may be limited due to technological constrains and complexity of the conversion processes under development. For these feedstocks there is not yet a SAF pathway that is commercially deployed.

Sugar and starch crops are more suitable for ATJ and SIP pathways, where starchy biomass such as sugarcane, maize, sweet sorghum, etc. are converted via fermentation into ethanol and then upgrade to jet fuels. Most of these feedstocks are easy to grow and with a long history of research and development thus available at commercial level (Figure 1-1). In fact, in some regions of the world, particularly in North and South America, feedstock such as maize and sugarcane are already used for biofuels production at commercial level, but not yet for aviation fuels.

The world leading producer of ethanol from maize and sugarcane are United Sates and Brazil, respectively. In fact, United States is the largest producer of maize around the world with about 33% of the total and large part of it dedicated to the production of ethanol. While Brazil concentrates about 41% of the global production of sugarcane. The pathway to convert these feedstocks into alcohol intermediates is well developed, which provides strong and fully mature technologies for the conversion of these kind of feedstocks into jet fuels (Table 3-5).



	ASTM Approved	4 CTD 4	f Supported ed by Mandates	Supported				Cost	t of Production	(§)		F	eedstock			Geopolitical/	
Routes		by Mandates		Max Drop in %	TRL	FRL	SAF Cost Dol- lar/Tonne	CAPEX %	OPEX %	Sustainability gCO2e/MJ	Туре	Availability (§§)	Cost Range (§§§)	SAF Fraction %	Geographical Concerns	References	
FT-SPK	2009	Voc	50%	69	7 8	1866 2250	69 92	14 179/	77 12 2	Waste and biomass	High	Low	40.70%	NO	[26.20_22]		
FT-SPK/A	2015	- ies	50-100%	- 0-0	/-0	1000-2200	00-03	14-17 70	7.7-12.2	residues	rugu	Low	40-7076	NO	[20,30-33]		
HEFA-SPK	2011	Only until 2030	50%	7-9	9	1100-1550	7–10	10-14%	13.9-60	Bio-oils, animal fat, Vegetable oils, UCO	Low	Med-High	20–55%	YES	[26,30–33]		
HFS-SIP	2014	Yes	10%	5–7	5–7	2100-2900	N/A	N/A	32.4–32.8	Sugarcane, sugar beet	Med	Med-High	90–100%	Yes	[26,30-33]		
ATJ- SPK	2016	Yes	50%	4–7	7	2100-2900	41-56	31-45%	23.8-65.7	Sugarcane sugar beet sawdust lignocellulosic residues (straw)	Med	Low	60–77%	Yes	[26,30–33]		
Co- Processing Bio-Oils in Petroleum	2018	Yes	5%	N/A	N/A	N/A	N/A	N/A	N/A	Fats, oils, and greases (FOG)	Low	Med-High	N/A	YES	[26,34]		
CHJ-SKA	2020	Yes	50-100%	6–7	6	N/A	N/A	N/A	N/A	Triglycerides such oils as soybean, jatropha and camelina Oil	Low	Med-High	33%	YES	[35]		
HC- HEFA-SPK	2020	Yes	10%	4	6	N/A	N/A	N/A	N/A	Algae	N/A	Med-High	N/A	NO	[35]		

Table 3-5: Feedstock availability and TRL level for the different SAF pathways (Shehab et al. 2023)

N/A refers to the lack of reliable data. § The CAPEX and OPEX are represented as a percentage of the total SAF cost per tonne. §§ The availability is described in three categories. *High* refers to enough available feedstocks; *medium* indicates average availability; and *low* refers to a limited feedstock. The data is based on Tables 1 and 2. §§§ The feedstock cost is described in three categories. *High* refers to an expensive feedstock that lies in the range of USD 300–2000; *medium* indicates an average cost in the range of USD 200–1500; and *low* refers to a cheap feedstock in the range of USD 50–300. The range of costs provided is a rough value and not reliable data, as the prices change significantly depending on market stability and demand.

Picarus

Table 3-6: Expected TRL level by 2030 of relevant advanced biofuel agricultural feedstocks. + TRL 3-5; ++ TRL 5-7; +++ TRL >7. Agricultural practices: intercropping (I), cover cropping (CC), rotation (R), agroforestry (AF), biochar (B). (Panoutsou et al. 2022)

		Relevant Biofuels (Sectors)	Agricultural Practices	Average Bas Crops and Lignocellulos Yields in Li	Average Baseline Yields (t/ha Seeds for Oil Crops and t/ha Dry Matter Biomass for Lignocellulosic Ones) per AEZ (in Parenthesis Yields in Land with Natural Constraints)		Productivity/Ability to Use Existing Machinery	Expected TRL by 2030 *
				Α	C and B	М		
	Rapeseed		I, CC, R, B	4.5 (2)	4 (2.5)	3 (1.5)	+++	+++
Oil	Ethiopian mustard	Hydrotreated	В	3.5 (1)	4 (1.5)	2.5 (1.5)	++	+++
	Crambe	vegetable oil	В	2 (0.5)	2.5 (1)	3 (1)	++	+++
	Camelina	 (HVO)/renewable diesel, HEFA 	I, CC, R, B	2.5	2.5	3 (1)	++	+++
	Cardoon	(aviation, marine,	В	3 (1.5)	3 (1.5)	3.5 (2)	+++	+++
	Safflower	neavy duty)	Ļ B	na	2 (1.5)	1.5 (0.8)	+	++
	Castor		Ļ B	na	na	3.5 (1.5)	+	++
	Willow		AF B	12 (9)	12 (9)	13 (8)	++	+++
	Poplar		AF, B	10 (8)	10 (8.5)	10 (7.5)	++	+++
	Biomass sorghum	Ethanol, methanol,	I, R, B	15 (9)	15 (9)	20 (12)	++	+++
losi	Tall wheat grass	butanol,	В	na	na	10 (7)	+	++
bellu	Miscanthus	Hydrotreated	В	12 (8)	15 (9)	20 (9)	+++	+++
gno	Switchgrass	bio-oil/biocrude	В	18 (10)	18 (10)	20 (12)	++	+++
Ľ	Cardoon	heavy duty)	В	14 (8)		20 (10)	++	+++
	Giant reed		В	15 (9)	15 (9)	20 (10)	++	+++
	Reed canary grass		В	15 (9)	15 (9)	20 (10)	++	+++

* Only crops with expected TRL by 2030 above 7 are further analysed in the section.



3.2 Technological innovations

The development of innovative cropping systems seeks to facilitate the supply of suitable feedstocks through the integrated production of food, feed and other biobased products in a sustainable manner, notably by valorising agricultural residues, increasing biomass yield, enhancing yield stability and sustainability, and optimizing feedstock suitability to SAF precursors (e.g., ethanol and isobutanol).

In that sense, innovative multiple cropping systems are defined as the cultivation and management practices used to grow two or more crops on the same land within the same growing period or immediately after a main crop. Cover crops (also called intermittent crops) for example, are grown during the winter and harvested in the spring before sowing of main crops. Multiple cropping systems (Figure 3-2) contribute to the intensification, in time and space, of the use of the land and its resources. This multiple cropping systems can be divided into: i) Sequential cropping systems; where two crops are grown in the same land but in a temporal sequence (i.e. the secondary crop is grown after the main one has been harvested); ii) Intercropping systems; where two or more crops are grown in the same land at the same time (i.e. in alternating rows of different crops; in strips running through the main crops; or in relay planting.)



Figure 3-2: Multicropping systems concept (source: FAO, 2012)

Producing biomass from multiple cropping systems, however, must balance main crops yield (i.e. food/feed crops), secondary crops yield (i.e. dedicated lignocellulosic crops), and the potential environmental benefits of such crop combinations (i.e. enhanced water infiltration; reduce soil degradation; fix atmospheric nitrogen and add organic matter to build soil quality; suppress weeds; store carbon dioxide). In fact, the introduction of a secondary crop requires a careful evaluation of its compatibleness with the main crop, site-specific pedo-climatic conditions, agronomic practices required. In any case trade-offs are inevitable in terms of feedstock quantity, quality, and management options.

In spite of their potential sustainability benefits, innovative multiple cropping systems, are still underutilized for SAF feedstock production purposes, but the additional biomass produced could become an incentive for farmers to introduce such practices (without affecting the main scope of agricultural systems to produce foo/feed) and increase their incomes.



Baldino et al. (2018) estimated the amount of cover crops that could potentially supply SAF in 2030. The most recent data on EU cover crops is reported in the 2010 Farm Structure Survey, which finds that just over 3% of arable land is planted with cover crops. Extrapolating from historical trends, Baldino et al. (2018) estimate that 10% of land used for annual crops in 2050 may be cover cropped and assume that half of this could be available for biofuel production without displacing fodder. This amounts to about 4.5 million hectares of cover crops that could provide an additional 7.15 Mt of lignocellulosic feedstocks for SAF production in 2030. The most common cover crops include low-starch legumes and grasses (i.e. Trifolium, ryegrass, oats). Moreover, there is some potential to use oilseeds such as rapeseed and carinata grown as cover crops, with ability to convert fuel through the existing, commercialized HEFA pathway (Del Gatto et al. 2015).

Some studies have evaluated the potential contribution of intercropping dedicated biomass crops to the sustainable development of advanced biofuels while improving biomass availability and yield stability. Zegada-Lizarazu et al. (2021a) for example quantified the impact of intercropping a dedicated lignocellulosic legume cover crop (sunn hemp) on the productivity of pearl millet and biomass sorghum (Figure 3-3). It was found that intercropping not only maintains the overall biomass production close to that of monocropped grasses potential, but can also lead to improved feedstock characteristics for determined bioenergy applications. For example, intercropped pearl millet resulted in improved mineral composition in terms of increased Si/K ratio and therefore potentially limited slagging problems in the reactors. In addition, intercropped sunn hemp showed increased cellulose content and a drastic reduction in mineral content, resulting in improved cell wall polysaccharide availability for biochemical conversion processes. Therefore, it was concluded that intercropping-dedicated lignocellulosic crops could be a feasible alternative for providing a mixture of feedstocks with improved qualitative characteristics while maintaining their biomass yield potential (Figure 3-3;

Table 3-7).





Sequential cropping systems (i.e. crop rotations, double cropping, relay-cropping) are characterized by growing compatible crop species in a temporal fashion in the same cultivated land and under the required agronomic management practices.



Table 3-7: Mineral concentration in several intercropping systems. (Zegada-Lizarazu et al. 2021a).

Mineral concentration of each cropping system and N level. * indicates statistically significant differences for each species among monocropped and intercropped systems and fertilisation levels. ns, no significant difference. CS, cropping system; PM, pearl millet; S biomass sorghum; SH, sunn hemp.

		Al	Ca	Fe	К	Mg	Na	Р	S	Si	Si/K	Ca/K
Cropping systems (CS)	Pearl Millet (PM)											
	Monocrop (PM)	17	2388	60	15540	2176	237	1937	1554	355	0.0234	0.1596
	Intercrop ($PM \times SH$)	36 ns	2452 ns	87 ns	13196 ns	2432 ns	296 ns	1678 ns	1542 ns	393 ns	0.0309 *	0.1895 ns
	Sorghum (S)											
	Monocrop (S)	13	1889	30	7729	1330	138	1021	738	378	0.0490	0.2461
	Intercrop (S \times SH)	29 ns	2006 ns	37 ns	8073 ns	1403 ns	150 ns	808 ns	679 ns	406 ns	0.0510 ns	0.2497 ns
	Sunnhemp (SH)											
	Monocrop (SH)	19	8547	58	10944	3390	229	1511	1548	270	0.0250	0.7894
	Intercrop (SH \times PM)	49 *	9627 ns	76 ns	10282 ns	3433 ns	334 ns	1495 ns	1519 ns	311 ns	0.0316 *	0.9560 *
	Intercrop (SH \times S)	41 ns	7492 ns	54 ns	9009 ns	2201 *	199 *	1146 ns	1204 ns	354 ns	0.0401 *	0.8401 ns
N level (N)	NO	28	4793	56	10927	2389	197	1436	1289	361	0.0363	0.4752
	N150	31 ns	5022 ns	60 ns	10522 ns	2288 ns	261 *	1309 ns	1227 ns	346 ns	0.0353 ns	0.5040 ns
	CS x N	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns

Mineral concentration is expressed as $mg kg^{-1}$.

These innovative systems can also reduce environmental concerns and provide additional feedstock for SAF production as demonstrated by several studies. It has been shown that in an innovative crop rotation scheme of sunn hemp with wheat or sugarcane the feedstock biomass was significantly enhanced with important positive ecosystem services on soil health and biodiversity, and without negative effects on food production.

Moreover, the qualitative characteristics (mineral, ash, and hemicelluloses contents) of the cumulated biomass feedstocks were higher than in the conventional system, suggesting that in temperate and tropical climates the integration of dedicated biomass legume cover crops within conventional food crops could lead to enhanced biomass availability, crop diversification, and efficient use (in space and time) of the land resources. (Zegada-Lizarazu et al. 2022).

Such results are somehow confirmed by other innovative cropping system studies. For example, double cropping seems a promising way to integrate food/feed crops and dedicated lignocellulosic crops. (Gesch et al. 2022) indicated that in a double-cropping study of sunflower after winter camelina in central Minnesota USA, the total oil yield (winter camelina + sunflower) was 1.5 times greater than its monocrop counterpart in one of the seasons of the study, and that the oil content and the oleic/linoleic acid ratio where similar to the monocrop controls. (Nafziger et al. 2016) found that four maize-based double cropping systems (i.e. maize-pannycress; maize-rye; maize-wheat) produced similar levels of energy (around 351 GJ ha⁻¹ of biomass energy). Whereas the soybean-based double cropping systems produced about 35% less energy than the maize-based systems, suggesting that despite the higher energy content of oil-rich seeds, higher biomass production systems lead to higher energy yields, despite the lower energy content of this biomass. In another study in Northern Italy, sunn hemp showed an attractive production potential when cultivated as a double crop with barley, wheat, or ryegrass. The energy produced by each double cropping system, in terms of kilocalories, followed the same trend as biomass production, with barley - sunn hemp showing the highest values and wheat - sunn hemp the lowest and ryegrass - sunn hemp with intermediate values suggesting variable degrees of complementarity with the growth cycle of the corresponding cereal crop (Zegada-Lizarazu et al. 2023); Figure 3-4). Therefore, double cropping could be considered a sustainable and feasible cropping system to integrate food/feed and dedicated biomass feedstocks.





Figure 3-4: Biomass energy [Ln(GJ ha-1) generated per cropping system. Numbers at the top and inside the bars are the back-transformed means (GJ ha-1). Different lowercase letters indicate significant differences among treatments. (Zegada-Lizarazu et al. 2023)

Another innovative strategy, among the sequential cropping systems, to improve land and resource use efficiency, to stabilize yields, boost biomass feedstock productivity per unit area, and promote biodiversity is the so-called relay-cropping, where a new crop is introduced into an already established one, resulting in an overlapping of the life cycles of the two crops. (Zegada-Lizarazu et al. (2021b) demonstrated that the sunn hemp - wheat relay cropping not only maintains stable the overall lignocellulosic biomass production of the system but can also lead to improved grain quality for bread-making wheat.

Location	Relay system	Crop biomass yield	Crop seed yield	Camelina seed yield	Energy output	Energy input	Net energy	Energy efficiency
		Mg ha⁻¹	kg ha⁻¹	kg ha⁻¹	GJ ha⁻¹			
Prosper, ND	Camelina-	10.9	-	1121	211.9	13.3	197.5	15.8
	Camelina-	6.4	1035	1484	71.7	12.2	59.6	5.9
	Camelina-maize	5.3	0	1531	37.9	15.6	22.3	2.4
Carringto n, ND	Camelina-	3.5	-	687	76.2	13.3	62.8	5.7
	Camelina-	2.7	494	775	35.7	12.2	23.5	3.0
	Camelina-maize	2.6	0	963	24.1	15.3	8.7	1.6
Morris,	Camelina-	16.2	-	239	278.8	13.3	265.4	20.8
IVIIN	sorgnum Camelina- soybean	-	1977	344	73.8	61.7	12.2	6.1
	Camelina-maize	9.4	3234	214	66.2	15.6	50.5	4.2

Table 3-8: Biomass and seed yield, and energy balance of different relay cropping systems at three locations in United States (modified from Berti et al. 2015)



Berti et al. (2015) indicated that the biomass sorghum relay planted with camelina showed a good potential for biofuel and energy feedstock production compared to maize and soybean in three locations of the northern Great Plains of United States (Table 3-8). Thus, rely on planting systems are able to provide food, feed, and fuel feedstock with low inputs and high energy efficiency.

3.3 Technological constraints

One of the main constrains to the production of SAF is the high feedstock costs which could lead to six times more expensive SAF than conventional jet fuels, which impacts the bankability of production facilities (EPRS | (2020).

Competition for feedstocks with other sectors, such as food, road transport, marine fuel, petrochemicals further complicate the economic feasibility of SAF, particularly when road transportation offers greater opportunities and revenues. Moreover, the location of the feedstock production sites and their proximity to conversion plants could affect the feedstock transportation costs and the stability of the supply. The feedstock supply limitation can somehow be overcome by establishing innovative cropping systems with enhanced biomass yields and therefore increased availability and steady supply.

HEFA production technologies are commercially available with the lowest production costs (estimated capital costs range from €0.40 to €1.50 per litre of annual capacity; (EPRS 2020). However, they have been associated with concerns over species' low yields, and therefore cost and availability of feedstock (Becken et al. 2023). Furthermore, HEFA feedstocks limitations increase when they are used as food-based materials or for road transport, which have simpler and less costly production process, and may therefore be a more attractive option to farmers.

Near term projections (2026) indicate that HEFA accounts for more than two-thirds of new capacity SAF production, potentially more than that if the aforementioned feedstock limitations of production cost and availability could be resolved (EPRS 2020; IEA 2022; Shehab et al. 2023). On the other hand, Fischer-Tropsch (FT) synthesis pathways, using lignocellulosic feedstocks, which are cheaper and with considerably higher biomass yield production potential (higher abundance), still needs to resolve issues related to the complicated and costly production process (i.e. ≤ 4 to ≤ 6 per litre of annual capacity). Thus, the limited availability of cost-effective and sustainable SAF feedstock and feedstock treatment infrastructures severely limits the development of SAF in the near future (EPRS 2020).

Although the demand for SAF is increasing, and besides the costs, the feedstock availability is still a major concern (Becken et al. 2023). The International Energy Agency's (IEA 2022.) Renewables 2022 report estimates restrictions on feedstock availability and supply for biodiesel, renewable diesel, and bio-jet fuel producers from 2022 to 2027 (IEA 2022) if feedstock production is not rapidly scaled up. To overcome the limitations on feedstock availability the IEA report has identified some potential solutions through for example the establishment of a wide ranging cooperation network including coordinating government actions, supporting the collection of agricultural residue feedstocks, promoting/funding the long-term development of innovative cropping systems, and strengthening synergies within the supply chains.

Currently, however, major consumer countries of biodiesel, renewable diesel and biojet fuels relay on their own selected/available feedstocks without any level of coordination. The EU, for example is phasing out the use of palm oil and is likely to limit the number of eligible feedstocks to wastes, agricultural residues, and rapeseed oil. Whereas United States will support the use of vegetable oils such as soybean for SAF production. Meanwhile, Indonesian biofuel manufacturers primarily use palm oil to produce biodiesel, and Brazilian rely on soybean oil (IEA 2022). Therefore, it could be said that the current feedstock sources and their availability are limited and there is a generalized lack of funding for research into the development of alternative feedstocks sources and sustainable crop production systems. Greater investment in production facilities, improve feedstock supply chains, seek out new



supplies and develop new feedstock production techniques are necessary to unlock future feedstocks availability limitations if the near-term supply targets are going to be meet.

Sustainability of feedstock production systems is also a major issue for SAF take off. Alternative feedstocks and their production systems need to incorporate sustainable production practices and standards, such as minimizing (direct & indirect) land-use change and protecting biodiversity. On the other hand, conventional feedstocks form agricultural crops such as corn, oil palm, soybean, or sugarcane, have been linked to increases in the cost of food and a range of adverse environmental and sustainability issues.

To overcome the limitations on agricultural crops the focus has shifted into dedicated lignocellulosic feedstocks (these are often by-products from agriculture such as crop residues, or dedicated annual and perennial lignocellulosic crops) and innovative cropping systems. It is important, however, that feedstock producers eliminate any potential competition with food production (i.e. increases in food prices due to competition for arable land), cause social issues such as displacement of local communities, labour exploitation, or pressure on primary forests or other valuable ecosystems (Malina et al. 2022; Becken et al. 2023; Folic 2023).

From the agronomic point of view, the development of innovative cropping system seems the most interesting alternative, although it still faces some important constrains because the implementation of multi cropping systems requires higher levels of farm organization, acquisition of new farmer skills, and development of diversified agricultural equipment and agricultural supplies suitable for the new crops and cropping system schemes. Therefore, farmers may initially be reluctant to implement such innovative cropping systems considering the higher cultural and management requirements and the need to acquire new skills.



4 Crop residues and waste streams

Another very important feedstock source for the production of SAF is the residues from crops and waste streams. According to RED II Annex IX A (as only advanced biofuels are addressed in the ReFuelEU Aviation) will be taken into account the following sources:

- a) Biomass fractions of mixed municipal waste, but not separated household waste for which recycling targets apply in accordance with point (a) of Article 11(2) of Directive 2008/98/EC.
- b) Bio-waste as referred to in point (4) of Article 3 of Directive 2008/98/EC from separately collected households under Article 3(11) of that directive.
- c) Biomass fractions of industrial waste not suitable for use in the food chain of humans and animals, including materials derived from retail and wholesale trade and from the agri-food industry.
- d) Straw.
- e) Animal manure and sewage sludge.
- f) Biomass fractions of waste and residues from forestry and related industries, i.e. bark, branches, leaves, needles, tree tops, sawdust, and shavings.
- g) Lignocellulosic materials other than logs and sawn wood.

Municipal solid waste and cellulosic waste can be processed into synthetic fuel through proven chemical reactions (i.e., the Fischer-Tropsch pathway) or converted into renewable isobutanol or ethanol and, further, into jet fuel through the "alcohol-to-jet" (AtJ) pathway. Other pathways are also under development. Due to its vast supply, there is great potential to use municipal solid waste as a sustainable feedstock. Rather than simply dumping municipal waste in a landfill site, where it will emit methane and other gases into the atmosphere, it can be used to create jet fuel. In the following paragraphs, an estimation of those sources' potential in EU will be presented.

4.1 Technological status (low TRL (long-term) and high TRL (shortterm)

4.1.1 Cereal Straw Potential

Straw is a by-product resulting from the growing of commercial crops, primarily cereal grain. EU-27 area under cereals amounts to almost 38.044.620 hectares and yields straw approximately 68.480.316 tones/year.

The traditional markets for straw include animal bedding, animal feed, and chopping and plough back to the soil to increase soil carbon content. Straw has been used in other EU countries for decades as a combustion fuel for both heat and electricity production.

Cereal production in the EU registered a negative sign in 2022, due to extensive drought that prevailed in the main agricultural countries of EU. According to Eurostat data, in 2022 the EU produced 270.9 million tonnes of cereals, 26.7 million tonnes less than in 2021, equivalent to a 9% decrease. In terms of the producing countries, Germany harvested 9.4 million tons of straw (13.9% of the EU total), Spain 8.8 million tons (12.9%), Poland 8.1 million tons (12%), and Romania 5 million tons (7.4 % of the EU total).

According to Eurostat data, in 2022 there were also countries with increased total grain harvest. The list includes Germany, which increased cereal grain production by 3% (1.1 million tons), Finland by 39% (1 million tons recovery after a poor harvest in 2021), and Poland by 3% (an increase of 1 million).

The basic use of straw is for animal feed. In order not to distort the feed market, for the scope of this report the estimated quantities that can be used as feedstock for SAF plants is the straw portion that remains unexploited.



4.1.1.1 Methodology

The estimation of the potential is a difficult process, due to the particularities it presents, and specifically to the difficulty of estimating and recording the elements of the raw material (quantity, availability) with precision and completeness.

The biomass categories examined in the context of this specific study are agricultural residues from winter grains (straw), pruning and forest residues. The above waste will be used as raw materials for the production of SAF and is in accordance with Annex XI of the amended Energy and Climate Directive (RED II).

More specifically, the methodology applied in this particular study followed the following stages:

- 1. Collection of data on the primary production (agriculture, forests) at EU27 level from Eurostat (year 2023)
- 2. Determination of the different types of biomasses, which will ensure the supply of the conversion technologies. For this project cereal straw and pruning were assessed.
- 3. Collection of data concerning quality characteristics of biomass raw materials such as calorific value per unit of dry product, moisture content, elemental and proximate analysis.

Using this raw data CRES estimated the potential residual lignocellulosic biomass that can be available from each crop type related to this project. In this way, the theoretical biomass potential is obtained with a high degree of accuracy.

Regarding pruning production in the EU27, it was based on literature whereas forest residues estimations are taken from Eurostat.

4.1.1.2 Results

According to our estimations, taking into consideration on the amount of cereal straw that is not actually exploited for feed production, Europe could technically produce more than 66 million tons of cereal straw per year that can be available for SAF production.

Among the major four cereals, wheat, oats, barley, and rye, the bulk of the availability of straw is anticipated for wheat straw and barley straw, which can reach 41.6 and 18.6 GT per year respectively, while oat and rye straw are following with estimated availabilities of 4.7 and 3.5 GT per year respectively (Table 4-1).

Wheat straw is mainly produced in France, where it can reach up to 8.9 million tons, followed by Germany, Poland and Slovenia with estimated availabilities of 4-5 million tons per year. Altogether these four countries reach more than half of the total wheat straw production in Europe.

Likewise, barley straw is mainly produced in Spain, France and Germany with 4.2, 3.2 and 2.8 million tons respectively per year.

Oat straw is mainly produced in Poland and Spain (1.3 and 0.8 million tonnes per year), which together account for half its production at EU level. Likewise, rye straw is mainly produced in Poland and Germany, with 1.36 and 1.13 million tons per year, respectively.



Table 4-1: Available straw for SAF production in EU Countries (Eurostat 2022)

	WHEAT T y ⁻¹	ОАТ Т у ⁻¹	BARLEY T y ⁻¹	RYE T y⁻¹
European Union - 27 Countries (From 2020)	41,604,876	4,764,366	18,599,832	3,511,260
Belgium	384,300	5,400	88,380	1,980
Bulgaria	2,187,000	0	248,400	14,400
Czech Republic	1,471,968	77,490	578,034	44,370
Denmark	880,344	105,696	1,010,268	195,498
Germany	5,191,200	267,840	2,895,660	1,131,840
Estonia	310,932	64,764	202,158	30,582
Ireland	100,260	48,582	335,196	0
Greece	559,458	112,572	214,164	14,040
Spain	3,510,720	829,278	4,237,020	228,528
France	8,981,856	269,118	3,256,938	69,516
Croatia		27,000	113,400	2,340
Italy	3,361,608	184,014	502,452	6,696
Cyprus	19,800	540	20,700	0
Latvia		177,480	156,420	59,040
Lithuania	1,703,160	180,000	291,420	51,300
Luxembourg	22,446	2,538	10,368	2,844
Hungary	1,896,444	39,240	742,392	52,002
Malta	0	0	0	0
Netherlands	0	0	64,422	4,230
Austria	507,564	34,092	220,878	73,350
Poland	4,366,314	1,313,622	1,153,980	1,364,166
Portugal	47,502	43,452	21,474	24,786
Slovenia	4,030,902	132,840	903,870	21,600
Slovakia	51,660	0	39,474	1,836
Finland	730,404	18,144	204,516	21,168
Sweden	427,140	569,700	636,120	48,240



In terms of the total straw production, France is leading the way with more than 12 million tons of straw per year, followed by Germany, Spain and Poland, the straw production of which overpasses the 8 million tons per year (Figure 4-1). Altogether, they account for more than half of the total cereal straw production in Europe.



Figure 4-1: Total cereal straw production in EU (in tons) in descending order

Straw is already widely used in energy generation applications, mainly as a fuel in specially designed boilers for the production of hot water or steam. Several other applications and conversion methods have been developed and tested, such as gasification and bioethanol production. Also, after a special pretreatment procedure, straw can be used for biomethane production through anaerobic digestion. Lately, straw along with other lignocellulosic agricultural residues is considered a potential feedstock for SAF production.

The following Table 4-2 presents the fuel characteristics of different types of straw as they are listed in the "Phyllis Database".

Feedstock	Moisture Content	Ash Content	Volatile matter, daf	Fixed Carbon, daf	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Halides	LHV	HHV
	wt%	wt%	wt%	wt%	wt%	wt%	wt%	wt%	wt%	mg/kg	Mj/kg	Mj/kg
CEREALS												
Barley	11.53	5.20	80.85	19.15	49.09	6.06	44.14	0.64	0.08	2.908.00	18.52	19.85
Corn Stover	6.06	4.75	85.17	14.83	49.31	6.04	43.56	0.70	0.11	2.803.00	17.75	19.06
Rice	11.73	17.79	82.18	17.82	49.15	6.23	42.13	1.59	0.13	7.803.60	17.11	18.47
Rye					49.64	5.85	44.16	0.25	0.04	624.90	18.49	19.78

Table 4-2: Lignocellulosic feedstock characteristics



To secure large-scale straw supply of satisfactory quality at reasonable prices, straw handling must be carried out as efficiently as possible. Straw producers and purchasers are still optimizing the different elements of the supply chains and organizing transport and storage efficiently. While most supplies are still in the form of big bales, optimization efforts have for instance resulted in an increased use of so-called midi-big bales, because these allow for more efficient road transport.

The handling of straw has developed into an independent discipline within agriculture, with heavyduty machinery primarily used by large farms and agricultural contractors. Since the 1980's, when the big balers hit the market, agriculture has invested considerable amounts of money in balers, rakes, front loaders, transport equipment, and storage facilities to be able to supply straws to the energy sector.

After harvesting, straw lies in swaths in the field, and the following handling elements are applied depending on the weather conditions and other factors.

- Raking
- Baling
- Pellets, briquettes, and chaffed straw
- Loading and unloading from trucks and lorries
- Field transport
- Decentral storage
- Loading for road transport
- Road transport
- Unloading at the plant
- Registration of weight and moisture content
- Buffer storage (at the plant)

Currently, there is a significant amount of expertise in dealing with straw management, and effective logistics and supply chain systems have been established. As a result, delivering straw is not a problem, provided that the required quantities have been secured.

4.1.2 Potential of other lignocellulosic agricultural residues

In the EU, a total of 11,301,345 hectares (ha) are dedicated to the cultivation of fruit trees (Figure 4-2). Olive orchards occupy the largest portion, with over 5 million hectares (45% of the total area), followed by vineyards, which cover more than 3 million ha (28%). Almond trees and other nut trees rank third, occupying over 1,200,000 ha (11%). Apple and pear orchards cover 611,540 ha (5%), while stone fruits (peaches, nectarines, apricots, cherries, and plums) cover 609,710 ha (5%). Citrus fruits account for 502,366 ha (4%). The remaining 141,000 ha (around 1.3%) are dedicated to figs, avocados, kiwis, other tropical fruits, and bananas.





Figure 4-2: Fruit tree area in the EU (Aliano-González et al. 2022)

Regarding the distribution of fruit-growing areas in EU member countries, Spain stands out with 43% of the total surface area, followed by Italy with 21%. Greece holds 10% of the fruit-growing area, while France accounts for 8%, and Portugal covers 6% (Figure 4-3).



Figure 4-3: Distribution of fruit tree areas in EU countries (Aliano-González et al. 2022)

Olives and grapes are the two major perennial crop systems traditionally grown in the Mediterranean Basin. Regarding olive production, Spain is by far the largest producer, responsible for over 50% of EU production, followed by Italy, Greece, and Portugal (Figure 4-4). Other leading olive-producing countries by annual metric tons are France and Croatia.



Figure 4-4: Distribution of olive trees and grapevines (Aliano-González et al. 2022)


Grapes are a significant cultural, economic, and ecological feature of the Mediterranean Basin, and they are also a cosmopolitan crop with the largest acreage and highest economic value among fruit crops globally. The EU members with the most extensive grapevine surfaces are Spain, France, and Italy (Figure 4-4).

The production of edible nuts in Europe has been gradually increasing since 2013. The most produced edible nuts in Europe are hazelnuts and almonds, with walnuts, chestnuts, and pistachio nuts produced to a lesser extent. Spain, the Netherlands, and Italy are major producers of processed edible nuts. Regarding primary production (growing, harvesting, and drying), the largest European producers are Spain (led by almonds), Italy (hazelnuts), Portugal (chestnuts), and France (walnuts) (Figure 4-5).

Thousands of varieties of apples are grown worldwide, many of which have been developed to thrive in various climates. This diversity has enabled commercial apple production in almost all EU member states. In 2019, Poland accounted for just over a quarter (26.6%) of the EU-27's harvested apple production. The other principal apple-producing member states were Italy (19.9%) and France (15.1%) (Figure 4-5).



Figure 4-5: Nut trees, apple and pear trees, stone fruit trees, citrus fruit trees, and other trees (Aliaño-González et al. 2022)

In contrast, the production of oranges and peaches is significantly restricted by climatic conditions, with about 93% of these crops produced in the EU-27 coming from Spain, Italy, and Greece.

Fruit tree pruning is a crucial process in orchard management. Its primary purpose is to shape the crown for optimal fruit production and efficient harvesting. Pruning is conducted at least once a year to maintain continuous and optimal production by controlling tree physiology. By trimming tree shoots, the number of fruits per plant is reduced, allowing for better nutrient distribution throughout the tree, effective canopy light exposure, and optimal bearing potential for regrowth, thus achieving quantitative and qualitative improvements in fruit crops.

There are considerable differences in wood production between different fruit trees and even within the same type of fruit tree. Wood waste production is influenced by factors such as crop type, variety and age, tree form, density, pruning type, climate and soil conditions, and other agronomic practices like irrigation. On average, annual pruning in favourable climatic and agronomic conditions can produce between 0.5 to 2.0 tons/ha of wood (dry weight, dw).

Given the current fruit tree area in Europe (11.33 million hectares), around 25 million tons of wood dry weight are produced annually from pruning. This wood must be removed to control diseases and facilitate future tending activities. Typically, it is either burned in-field or crushed onto the soil, resulting in no direct economic benefit.

Collecting wood from pruning presents logistical challenges due to several factors: (i) its dispersion across territories, (ii) the size and layout of plantations, and (iii) the relatively low biomass production per hectare compared to forestry wood. However, this waste is an excellent biomass source. Being a



residual material, it does not create additional land demand and can substantially reduce GHG emissions compared to fossil fuels, decreasing pressure on fossil fuel reserves and energy dependence. Pruning residues can be used as feedstock for sustainable aviation fuel (SAF) production through proven chemical reactions (e.g., the Fischer-Tropsch pathway) or converted into renewable isobutanol or ethanol and further into jet fuel through the "alcohol-to-jet" (AtJ) pathway (Aliaño-González et al. 2022).

Table 4-3 presents the fuel characteristics of different types of trees as they are listed in the Phyllis Database, whereas Table 4-3 presents the fuel characteristics of different types of trees as they were measured in CRES fuel characterisation laboratory

Feedstock	Moisture Content	Ash Content	Volatile matter, daf	Fixed Carbon, daf	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Halides	LHV	HHV
	wt%	wt%	wt%	wt%	wt%	wt%	wt%	wt%	wt%	mg/kg	Mj/kg	Mj/kg
TREES												
Olive tree pruning	6.30	5.43	86.52	13.48	51.91	6.26	46.82	1.06	0.10	859.9	19.75	20.15
Vineyard pruning	9.13	8.61	73.51	26.49	50.31	5.56		1.17	0.09	348.3		20.13
Orange tree	31.09	3.02	84.66	15.34	48.58	5.53	44.72	1.08	0.06		17.70	18.91
Almond tree	11.40	1.67	80.52	19.48	50.11	6.03	43.22	0.63	<0.05		19.84	18.53

Table 4-3: Fuel characteristics of feedstock from trees.

Table 4-4: Tree pruning fuel characteristics

Feedstock	HHV db	LHV db	Volatiles	Ash	Fixed Carbon	C	Н	N
	Mj/kg	Mj/kg	%	%	%	%	%	%
APPLE	19.13	17.84	77.40	5.27	17.34	46.82	6.22	1.28
PEAR	19.57	17.84	76.90	3.73	19.38	47.28	6.15	1.17
CHERRY	19.37	18.05	75.30	3.60	21.10	51.42	6.06	0.36
PEACH	19.82	18.51	77.39	3.56	19.05	48.17	6.20	1.08
APRICOTS	19.47	18.14	77.47	4.34	18.20	47.05	6.29	0.87
OLIVE TREES	20.36	18.99	80.38	5.78	13.85	47.92	6.33	1.21
OLIVE TREES noL	19.36	17.90	78.92	3.68	17.40	46.99	5.72	0.79
GRAPES	19.08	17.74	77.64	3.63	18.64	47.20	5.98	0.70



Agricultural pruning has the potential to be used extensively for energy production, but its utilization is limited due to several constraints such as high costs and lack of technology. The availability of constant demand is the primary driving force for establishing a reliable supply chain for tree pruning material. Numerous studies have indicated that the pruning collection stage carries the highest cost. Therefore, cost-efficient biomass processing and supply play a crucial role in strategically planning and implementing an economically viable pruning supply chain.

4.1.3 Potential of lignocellulosic residues from forestry

Historically, wood has been the major biomass source for energy use. Today, fuelwood, wood pellets, and wood chips are often used for space heating and power generation within the developed world. Wood for use as an energy source (a fuel) comes not only from tree felling but also from selective thinning of managed forests and other forestry practices (direct sources). The main wood-based products are (i) cork and wood, (ii) pulp and wastepaper, (iii) cork and wood manufacturers (excluding furniture), and (iv) paper, paperboard, and by-products. Wood for energy use may also be derived as a by-product from downstream processing in wood-based manufacturing, for example, as off-cuts, trimmings, sawdust, shavings, wood chips, or black liquor (indirect sources). End-of-life wood and paper products may also be used as a source of energy (recovered wood).



Figure 4-6: Classification of the wood-based fuels according to CEN/TS 14961. Source: VTT

Figure 4-6 shows all the routes of forest wood utilisation including the uses for energy purposes. As it can be seen from this Figure most of the residues coming out from the indirect use of forest wood can be used for biofuel production. This is the reason that is important to estimate the potential of woody biomass derived from forests.

According to Eurostat the total wooded area of EU-27 countries is 159,725,220 ha. The distribution among the 27 countries is shown in

Table 4-5.



Table 4-5: Wood area of EU-27, ha

Data extracted on 12/03/2024 16:08:53	3 from [ESTA	T]								
Dataset:	Area of wooded land (EFA questionnaire) [FOR_AREA									
Last updated:	18/12/2023	23:00								
Time frequency		Annua	al							
Stock or flow		Closir	ng stock							
Forestry indicators		Fores	t							
Unit of measure		Thous	and hectares							
TIME	2000		2020		2021					
GEO (Labels)										
European Union - 27 countries (from	:		159.555,79	S	159.725,22	S				
Belgium	667,3	s	689,3	s	689,3	S				
Bulgaria	3.375	s	3.896		3.897					
Czechia	2.637,29	s	2.677,09	s	2.678,9	S				
Denmark	571,6	S	628,44	s	628,44	S				
Germany	11.354	s	11.468,09		11.474,87					
Estonia	2.238,89	s	2.438,4	S	2.438,4	S				
Ireland	630,36	s	799,14		808,85					
Greece	3.600,23	S	3.901,8	s	3.901,8	S				
Spain	17.093,93	s	18.572,17	S	18.576,46	S				
France	15.288	S	17.489,13	е	17.570,82	е				
Croatia	1.885	s	1.940		1.943,4					
Italy	8.369,25	s	9.566,13	s	9.619,94	S				
Cyprus	:		172,54		171,73					
Latvia	3.241	S	3.410,79	S	3.414,66	s				
Lithuania	2.020	S	2.202,19		2.202,19	S				
Luxembourg	86,7	S	88,7		88,7					
Hungary	1.921,17	S	2.053,01	s	2.051,55	S				
Malta	0,35	S	0,46	S	0,46	S				
Netherlands	359,5	S	363,8		363,8	S				
Austria	3.838,14	S	3.886,55	р	3.889,09	р				
Poland	9.059	S	9.464,2		9.467,5					
Portugal	3.281	S	3.340,63		3.343,34					
Romania	6.366	S	6.981,62		6.981,66					
Slovenia	1.233	S	1.185,13		1.180,6					
Slovakia	1.901,41	S	1.951,49		1.952,76					
Finland	22.445,64	S	22.409	S	22.409	S				
Sweden	28.163	S	27.980	S	27.980	S				

Respectively, the total available volume of timber is 28,539,588,910 cubic meters, which corresponds to 19,977,712 ktons if we use an average wood density of 700 kg/m³. The availability of timber per EU country is shown in Table 4-6.

The latest Report for the State of the Energy Union, that the Commission presented to the European Parliament, the Council, the European Economic and Social Committee, and the Committee of the Regions in 2023, states that in the EU, woody biomass is the main feedstock reported for solid biomass production (labelled as "forest biomass" in Figure 4-7, accounting for 66% of the total and followed by biomass from organic waste (26%) and agricultural biomass (8%). Germany records a significant production of organic waste biomass (137,675 thousand m³). It also records production of the largest share of forest biomass (66,658 thousand m³) in the EU, followed by Sweden (65,102 thousand m³). Spain records the highest volumes of agricultural biomass (20,844 thousand m³).



Table 4-6: Volume of timber for the EU-27 countries, thousand m³

Data extracted on 12/03/2024 16:14:02	2 from [ESTA	T]				
Dataset:	Volume of ti	mber	over bark (EF	A que	stionnaire) [F	OR_V
Last updated:	18/12/2023	23:00				
Time frequency		Annua	al			
Stock or flow		Closir	ng stock			
Forestry indicators		Fores	t			
Unit of measure		Thous	and cubic m	etres		
TIME	2000		2020		2021	
GEO (Labels)						
European Union - 27 countries (from	21.695.624,09	S	28.333.751,85	S	28.539.588,91	S
Belgium	155.796,3	S	181.937,16	S	182.396,11	S
Bulgaria	556.618,31	S	718.410		725.630	
Czechia	692.797,93	S	770.855,89	S	759.987,22	S
Denmark	102.588,76	S	138.295		140.360,33	S
Germany	3.251.762,37	S	3.798.214,18		3.791.584,13	
Estonia	428.660	S	513.233,93	S	514.130,74	S
Ireland	56.016,85	S	128.950,2		142.045,9	
Greece	154.219,94	S	203.331,76	S	205.239,3	S
Spain	890.284,27	S	1.329.683,31	S	1.347.711,47	S
France	2.035.181,83	S	3.341.607,07	S	3.386.334,57	S
Croatia	346.245,78	S	430.108,1		433.278	
Italy	969.541,58	S	1.470.933,93	S	1.489.012,28	S
Cyprus	7.930	S	12.061,06		12.250,9	
Latvia	552.248,67	S	674.427,93	S	677.804,8	S
Lithuania	434.098,57	S	566.700		573.070,75	S
Luxembourg	28.011,51	S	35.050,55		35.593,77	
Hungary	326.904,52	S	391.158,13	S	392.543,04	S
Netherlands	58.330,02	S	82.043,82		82.346,76	S
Austria	968.814,42	S	1.220.568,3	р	1.225.089,96	р
Poland	2.015.207,61	S	2.668.958,5		2.678.366,5	
Portugal	129.710,79	S	181.204,65	S	181.834,12	S
Romania	1.654.513,65	S	2.409.952,99		2.444.212,11	
Slovenia	339.323,08	S	356.700,4		359.810,21	
Slovakia	465.944,35	S	542.666		545.800	
Finland	1.904.371,62	S	2.566.701,79	S	2.594.507,89	S
Sweden	3.170.501,38	S	3.599.997,19	S	3.618.648,05	S

Forest biomass was the largest reported category across all the Member States (262,858 thousand m3). Germany reported 12% of the total reported primary supply of solid biomass from forest, followed by Spain and Poland (both reported 11%), and Sweden and France (both reported 10%). The second largest reported category of supply of solid biomass was municipal waste (171,023 thousand $m^3 - 24\%$ of the total). Germany reported 74% of the total renewable municipal waste, followed by Sweden (8%), Belgium (6%), Spain and the Netherlands (both reported 4% of the total), Italy (2%) and Austria and Portugal (both reported 1% of the total). The third largest reported category for primary supply of solid biomass was forest-based industry co-products (144,821 thousand $m^3 - 20\%$ of the total). Sweden reported 22% of the total reported forest-based industry co-products, followed by Finland (20%), Austria (11%), Germany (10%), France (6%), Poland (5%), Estonia (4%) and Latvia (4%).

Overall, primary supply of solid biomass in the EU has increased from 3,336,811 TJ in 2008 to 4,454,768 TJ in 2021, an increase of 33.5% overall. In 2021, when it comes to the Member States individually, Germany was the EU's biggest producer of solid biomass (767,891 TJ), followed by France (530,659 TJ), Sweden (460,620 TJ), Poland (377,690 TJ) and Finland (352,535 TJ). Austria follows with 250,710 TJ, Estonia with 104,208 TJ and Greece with 33,317 TJ. Based on the reported data, in Germany the largest share of solid biomass came from renewable municipal waste (125,984 thousand m³). The other Member States report mainly forestry-based solid biomass, often not distinguishing between energy and material use. Member States collectively reported that roundwood is the largest category of forestry-based solid biomass (215,440 thousand m³), followed by fuelwood (176,304 thousand m³) and renewable municipal waste (171,023 m³).





Figure 4-7: Primary supply of solid biomass in 1000 m3 for energy production, indigenous production in 2021 grouped by feedstock origin (EC, 2024)

The fuel characteristics of different types of forestry biomass are presented in Table 4-7. Data is taken from the Phyllis Database.

Feedstock	Moisture Content	Ash Content	Volatile matter, daf	Fixed Carbon, daf	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Halides	LHV	HHV
	wt%	wt%	wt%	wt%	wt%	wt%	wt%	wt%	wt%	mg/kg	Mj/kg	Mj/kg
FORESTRY												
Pine tree	3.87	0.58	80.48	19.52	52.11	6.14	41.44	0.30	0.01	42.30	19.68	21.02
Eucalyptus	9.80	0.72	86.69	13.31	49.19	6.05		0.17	0.02		18.46	19.78
Oak			82.30	17.70	48.62	6.03	44.87	0.12	0.01	101.70	17.94	19.25

Table 4-7: Forestry wood feedstock characteristics.





Figure 4-8: Logging residues valorisation (VTT)

The process of recovering waste wood from various sources such as municipal waste or different industries is relatively straightforward. The timber processing industry usually collects every waste generated during their operations, which is then utilized by the same industry for energy production. Additionally, wood from municipal waste is either recovered on-site through recycling schemes or at landfills through mechanical waste separation plants.

The tricky case is to recover wood residues that are left in the forest during the cutting process. Depending on the morphology of the forest this recovery process may be very difficult. Nevertheless, counties with developed forestry industry, have established efficient collection processes that recover this type of residues from the forests. Figure 4-8 from VTT, illustrates the process of collecting forestry residues in Finland.

4.1.4 Potential of Dried Sewage Sludge

Dried sewage sludge can be also used as a potential feedstock for the production of SAF through thermochemical pathways. Sewage sludge or residues from industrial processes are wastewaters with high organic content. Depending on the previous treatment that sludge has been undergoing, it has considerable energy content. This content can vary from 19.75 kJ/kgDS to 13.60 kJ/kgDS as can be seen in *Table 4-9* (Gorgec et al. 2016)



Table 4-8: Sewage sludge production from WWTP in thousands of tons (EUROSTAT 2024)

Dataset:	Sewage sluc	ge production and di	sposal from urban wa	stewater (in dry substar	nce (d.s)) [ten00030]								
Last updated:	03/01/2024 2	23:00											
Time frequency	Α	nnual											
Unit of measure	Т	housand tonnes											
Wastewater treatm	nent plant para S	Sludge production - tot	al										
ТІ	ME 2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	
Belgium	160,56	е :		: :	:	:	:	:	:	:	:		:
Bulgaria	49,80	51,40	59,30	60,30	54,90	57,40	65,80	68,60	53,10	44,43	:		:
Czechia	196,30	217,90	263,30	260,10	238,59	210,24	206,71	223,27	228,22	221,09	219,11	235,10)
Denmark	141,00	:		: :	:	:	:	:	:	:	:		:
Germany	1.893,64	1.946,29	1.848,8	1.808,72	1.830,82	1.820,57	1.794,36	1.785,55	1.761,62	1.749,86	:		:
Estonia	18,80	18,30	21,70	17,00	19,91	19,11	18,65	20,94	25,54	19,48	18,99	20,22	2
Ireland	89,99	85,65	72,43	64,55	53,54	58,39	56,02	58,77	55,23	58,63	58,45	60,47	7
Greece	:	147,00	118,62	113,04	116,11	119,77	119,77	103,28	103,28	103,28	98,55	98,55	5
Spain	1.355,10	1.331,60	1.233,40	1.122,60	1.131,60	1.152,60	1.174,40	1.192,00	1.210,40	:	:		:
France	1.025,00	1.022,00	1.043,00	909,00	1.059,00	1.238,00	1.006,00	1.174,00	:	:	1.092,90		:
Croatia	30,30	31,00	16,9	5 16,02	16,31	b 17,94	19,72	17,60	19,23	20,65	21,71	27,46	6
Italy	1.102,70	:		: :	:	:	:	:	:	:	:		:
Cyprus	7,08	6,82	6,5	6,12	6,16	6,70	7,41	7,17	8,41	8,68	8,22	8,83	3
Latvia	25,24	19,91	20,14	22,93	22,32	22,48	26,65	25,62	25,14	25,09	23,27	18,99	9
Lithuania	51,31	51,83	45,09	41,43	40,71	44,45	44,42	42,49	44,19	39,94	41,05	39,63	3
Luxembourg	9,70	:	8,68	3 :	:	9,16	8,92	e 9,32	e 9,08	e 8,89	e 9,47	e 9,36	6
Hungary	170,34	168,33	160,60	170,47	163,12	177,70	217,96	266,84	233,66	227,89	167,03	226,21	1
Malta	1,24	6,06	b 10,50	9,64	8,50	8,44	10,77	10,30	8,28	9,69	10,36	10,37	7
Netherlands	351,00	350,80	346,40	339,10	345,00	354,60	347,60	:	341,77	:	353,85		:
Austria	262,80	:	266,30) :	239,04	:	237,94	:	234,48	233,56	228,01	193,62	2
Poland	526,70	519,20	533,30	540,30	556,00	568,00	568,33	584,45	583,07	574,64	568,86	584,75	5
Portugal	:	:	338,80) :	85,89	:	119,17	:	:	:	:		:
Romania	82,10	114,10	85,40	172,80	192,33	210,45	240,41	283,34	247,76	230,59	254,22	264,34	1
Slovenia	30,10	26,80	26,20	27,20	28,30	29,10	32,80	36,70	38,10	34,80	31,00	27,48	3
Slovakia	54,76	58,72	58,7	57,43	56,88	56,24	53,05	54,52	55,93	54,83	55,52	54,76	6
Finland	142,70	140,90	141,20	95,20	115,70	146,00	146,99	161,19	146,62	160,17	153,65		:
Sweden	203,50	200,10	207,50	207,90	200,50	197,50	204,30	205,60	211,60	212,80	208,30	204,80)



Type of Sludge	Solids Content %DS	Energy Content kJ/kgDS
Primary sludge	3.5	19,750
Undigested blended (P+S) sludge	5.0	13,450
Sludge after digestion	3.3	12,400
After thermal drying*	95	13,600

Table 4-9: Calorific Values from waste water treatment plants – Low heating value.

*50% biological sludge digested.

Sludge is produced during the wastewater treatment processes. Wastewater consists of up to 99% water with the rest being solids, dissolved and particulate matter, microorganisms, nutrients, heavy metals, and micropollutants, although the exact composition obviously differs depending on the source. Domestic and municipal wastewater is likely to contain high bacterial loads, whereas industrial activities can produce wastewater that is characterised by a broad spectrum of pollutants.

The treatment of wastewater is not a single-step process and consists of several separate and sequential stages that rely on a combination of physical, chemical and biological processes to remove contaminants. There are several levels of wastewater treatment, the choice of which depends on the type of contaminants, the pollution load, and the discharge requirements, as well as the anticipated end use of the effluent. Treatment that is tailored to produce effluent of a quality that meets the needs of the intended end-uses is known as "fit-for-purpose" treatment. The number of treatment technologies is vast and includes physical, chemical and natural processes. The aim of wastewater treatment is that the treated water or effluent is clean enough either to be safely used again or to be returned to the water cycle with minimal environmental impact.

4.1.5 Potential of food waste from MSW

Municipal solid waste can be a potential source of feedstock for the production of SAF. Carbon-based waste such as product packaging, grass clippings, furniture, clothing, bottles, food scraps and newspapers can be properly processed and converted into SAF. The valorisation of food waste from households towards the production of advanced biofuels is also predicted in the Renewable Energy Directive (RED II Annex IX A). Therefore, it is crucial to investigate the available potential of food waste that can be recovered from MSW, as this source is vast and practically endless.

The MSW generation in Europe from 2004 and 2022 is presented in Figure 4-9. Municipal waste generation totals vary considerably, ranging from 301 kg per capita in Romania to 835 kg per capita in Austria. The variations reflect differences in consumption patterns and economic wealth, but also depend on how municipal waste is collected and managed. There are differences between countries regarding the degree to which waste from commerce, trade and administration is collected and managed together with waste from households. As it can be seen there is an increasing trend in MSW generation, which justifies the importance of this source of potential SAF feedstock.



Municipal waste generated, 2004 and 2022

(kg per capita)



Note: countries are ranked in increasing order by municipal waste generated in 2022. ⁽¹⁾ 2021 data. ⁽²⁾ 2020 data. *Source*: Eurostat (online data code: env_wasmun)

eurostat O

Figure 4-9: Municipal solid waste generated in Europe in 2004 and 2022.

Another fact that supports the case of MSW is that though the recent years an increasing amount of MSW are properly treated and most of the useful fraction of them are recovered, leaving only a small portion for landfilling.

Even though more waste is being generated in the EU, the total amount of municipal waste landfilled has diminished. The total municipal waste landfilled in the EU fell by 69 million tonnes, or 56 %, from 121 million tonnes (286 kg per capita) in 1995 to 53 million tonnes (118 kg per capita) in 2022. This corresponds to an average annual decline of 3.1 %. For the shorter period 2004-2022, landfilling fell by 3.2 % per year on average. As a result, the landfilling rate (landfilled waste as share of generated waste) in the EU dropped from 61 % in 1995 to 23 % in 2022.

Respectively, the amount of waste recycled (material recycling and composting) rose from 37 million tonnes (87 kg per capita) in 1995 to 111 million tonnes (248 kg per capita) in 2022 at an average annual rate of 4.0 %. The share of municipal waste recycled overall rose from 19 % to 48 %.

Waste incineration has also increased steadily in the reference period, though not as much as recycling and composting. Since 1995, the amount of municipal waste incinerated in the EU has risen by 29 million tonnes or 98 % and accounted for 59 million tonnes in 2022. Municipal waste incinerated has thus risen from 70 kg per capita to 133 kg per capita. Table 4-10 shows the amount of municipal waste treated in the EU for the period 1995 to 2022 by treatment method, in million tonnes and in kg per capita.



Table 4-10: Amount of municipal waste treated in the EU for the period 1995 to 2022

Municipal waste landfilled, incinerated, recycled and composted, EU, 1995-2022

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Change 2022/1995 (%)
															millior	n tonne	s												
Landfill	121	117	117	114	113	112	107	104	99	93	88	88	87	83	82	79	74	67	63	59	57	54	53	53	55	54	52	53	-56
Incineration	30	30	33	33	34	36	37	39	39	41	45	48	49	51	52	53	55	54	56	57	57	58	59	59	59	62	62	59	98
Material Recycling	23	26	30	32	37	38	40	43	43	43	46	47	52	53	54	55	56	58	56	59	63	65	66	67	67	69	72	68	196
Composting	14	16	17	18	19	23	23	24	24	26	26	27	28	30	30	29	29	30	31	33	33	36	38	38	39	43	46	43	203
Other	10	13	12	11	12	11	12	12	12	13	16	13	11	10	7	6	6	6	5	4	4	5	4	5	5	4	6	6	-37
															kg pe	r capita													
Landfill	286	276	276	266	263	262	250	241	229	215	202	202	199	190	186	178	167	153	142	134	127	121	120	119	124	121	117	118	-59
Incineration	70	71	77	78	79	84	87	90	90	95	103	111	112	116	117	121	125	122	127	128	128	131	133	132	131	138	138	133	91
Material Recycling	54	62	69	75	85	87	92	100	100	100	105	109	119	120	123	125	128	130	128	134	141	146	148	149	150	154	162	153	181
Composting	33	38	41	42	45	53	54	57	57	59	59	61	64	69	67	66	66	69	71	73	75	82	85	85	87	96	102	96	187
Other	23	31	28	27	28	27	27	27	28	31	37	30	24	23	17	13	13	13	11	9	9	10	10	14	12	9	13	14	-40

Note: data estimated by Eurostat. Source: Eurostat (online data code: env_wasmun)

eurostat O



The evolution of MSW treatment in Europe from 1995 till 2022 is shown in Figure 4-10. Especially for food waste the total amount produced in EU-27 for 2020 is shown in Table 4-11.



Municipal waste treatment, EU, 1995-2021

Note: estimated by Eurostat. Source: Eurostat (online data code: env_wasmun)

eurostat 🖸

Figure 4-10: Municipal solid waste generated treatment in Europe 1995-2022



Table 4-11: Food waste generation for 2020 (EUROSTAT)

BIO FOOD WASTE		Total (aggregate changing according to the context)	Food waste - bio, household and similar waste
GEO (Labels)	Capita	kg per capita	t
European Union - 27 d	countries (from 2020)	130	55.820.322
Belgium	11.629.213	250	2.907.303
Bulgaria	6.520.314	108	704.194
Czechia	10.524.167	91	957.699
Denmark	5.873.420	221	1.298.026
Germany	84.432.670	131	11.060.680
Estonia	1.328.439	125	166.055
Ireland	5.011.500	154	771.771
Greece	10.678.639	191	2.039.620
Spain	47.326.687	90	4.259.402
France	68.128.000	129	8.788.512
Croatia	4.036.355	71	286.581
Italy	58.929.360	136	8.014.393
Cyprus	888.000	397	352.536
Latvia	2.003.000	145	290.435
Lithuania	2.860.002	137	391.820
Luxembourg	645.397	147	94.873
Hungary	9.730.772	93	904.962
Malta	516.100	154	79.479
Netherlands	17.641.147	161	2.840.225
Austria	9.027.999	136	1.227.808
Poland	38.039.000	112	4.260.368
Portugal	10.344.802	176	1.820.685
Slovenia	2.120.937	68	144.224
Slovakia	5.447.000	106	577.382
Finland	5.541.000	116	642.756
Sweden	10.545.310	89	938.533
TOTAL			55.820.322



4.2 Technological innovations

The collection of biomass residues is still expensive or challenging, taking into account the high variability of the types of the residues (straw, tree pruning, forest residues), their dispersed geographic allocation leading to high transportation costs, their seasonal availability and limited harvesting window requiring time-efficient biomass collection systems.

On the other hand, the large bioenergy plants for SAF production need consistent supply of large volumes of biomass throughout the year, with a consistent fuel quality.

Technical innovations on agricultural residues focus on:

- Feedstock collection

Branches and shoots that have been pruned are usually left on the field and are neither chipped nor used for energy production. They are either simply removed from the orchard and burned at the side of the field, or are mulched and incorporated with the soil, as soil improvement.

Efficient methods need to be developed for collecting the diverse biomass residues directly from fields or forest sites, which must be cost-effective, environmentally friendly, that will maintain soil health and at the same time optimize the residual biomass production. This involves the development of specialized equipment and techniques tailored to different types of biomasses, be it fruit tree pruning, vineyards or olive tree pruning. Only recently experimentation on their collection and handling to build cost-efficient supply chains for their energy exploitation has started (EU projects: Agroinlog, Europruning, uP_running, Music, Agrochains – a Greek project).

- Biomass handling and processing equipment optimization

Raw biomass has low energy density, which often needs densification to facilitate transportation and storage. This can be achieved with the production of intermediate energy carriers – as discussed in the following chapter.

- Strategies for mobilizing biomass residues effectively and building integrated biomass supply chains.

Innovative best practices regarding the mobilization of residues and wastes, to minimize transportation costs during several stages in the biomass supply chains are required to maximize the cost and quality efficiency of the supply chains. This includes route optimization, scheduling, and coordination of biomass collection, storage, and delivery activities. Enabling technologies, such as digitalization, is essential to improve feedstock mobilization at the regional and local levels.

4.3 Technological constraints

High variability of the types of the residues (straw, tree pruning, forest residues) with different fuel characteristics, requiring different harvesting/collection equipment

Dispersed geographic allocation leading to high transportation costs

Location of the feedstock production site and its proximity to conversion plants --> transportation costs

Seasonal availability and limited harvesting window requiring time-efficient biomass collection systems

High feedstock prices and limited availability and scalability of sustainable feedstock



5 Bioenergy carriers

This section explores the dynamic landscape of sustainable aviation fuel (SAF) production, emphasizing three key bioenergy carriers. The investigation into non-conventional oleaginous yeasts uncovers pivotal environmental conditions for optimizing microbial oil (MO) yields. A review on isobutanol production strains unravels genetic modifications and environmental influencers crucial for enhancing efficiency during sugar fermentation. An assessment of syngas production, shedding light on advanced gasification techniques, gas conditioning methodologies, and upgrading approaches is also presented. This comprehensive exploration aims to yield valuable insights into the technological advancements and challenges within these bioenergy carriers, conFtributing to the evolution of sustainable aviation technologies.

5.1 Technological status - low TRL (long-term) and high TRL (short-term)

5.1.1 Non-conventional Oleaginous Yeasts for Microbial Oils (MO) Production:

An organism qualifies as oleaginous when it possesses the ability to store a minimum of 20% of its dried biomass as internal lipids. This characteristic is observed in approximately 70 yeast species, several of which exhibit noteworthy traits enabling them to accumulate up to 40% lipids. Under optimized conditions, some of these species can even achieve lipid concentrations as high as 70% or even 80% (Robles-Iglesias et al. 2023).

These microorganisms are also commonly referred to in the literature as single cell oils (SCOs). Their remarkable capabilities position SCOs as promising microbial platforms for lipid production, characterized by a high percentage of fatty acids (FAs), derived from various feedstocks. Essentially, SCOs serve as bioenergy carriers that can be used as biological catalysts to extract FAs for biofuel production (Lopes da Silva et al. 2023).

The diversity of available yeasts for this purpose has led to a dispersion of research efforts, resulting in variations in the developmental stages of different species. According to Robles-Iglesias et al. (2023), the overwhelmingly predominant focus of research in the realm of oleaginous yeasts is *Yarrowia lipolytica*, with other strains being categorized as non-conventional for the purposes of this report Robles-Iglesias et al. 2023)).

Table 5-1 presents a non-extensive review on Technological readiness Level (TRL) of research on different non-conventional yeast species. It includes examples of reactor conditions, and the results obtained from these experiments.



Table 5-1: Review on TRL for non-conventional yeasts

Yeast Species	Conditions	Results	TRL (Technology Readiness Level)	Reference
Lipomyces starkeyi	3 L batch. Expired glucose 50 g/L, pH 6,0, T = 28 ºC,	Total max Dry cell weight of 34.0g/L, containing lipids at 34.1% w/w (SCO=11.6g/L). Lipids mainly composed of triacylglycerols.	TRL 4	Diamantopoulou 2023
Rhodosporidium toruloides	7 L Fed-batch. Secondary Brewery wastewater plus sugarcane molasses. 143h.	42,48 g/L max biomass concentration; 11,02 g/L TFAs max concentration. 81,82% COD removal. A different medium with glucose for 260h produced 126,84 g/L max biomass and 28,51 g/L TFAs.	TRL 5	Dias, 2020
Rhodosporidium toruloides	1500 L Fed-batch. sugarcane juice and urea.	Economically competitive biodiesel (0,76 USD/L).	TRL 7	Soccol 2017
Yarrowia lipolytica (genetically modified)	3 L fed-batch reactor. Glucose 100 g/L, pH 6,8, T = 28 ºC.	High yield (0.252 g/g, 93% of theoretical yield), productivity (0.97 g/L/h), and oil content (81.4% in bioreactor)	TRL 4	Xu 2017
Rhodotorula glutinis	500 L fed-batch. Crude glycerol 40 g/L (by- product from biodiesel production)	Maximum concentrations of biomass, lipids, and carotenoids obtained were 46.32 g/L, 37.65%, and 713.80 mg/L, respectively.	TRL 7	Sriphuttha 2023
Trichosporon oleaginosus	250 mL medium. 40 g/L glucose, 1g/L Ammonium sulfate, C/N ratio 76	maximum lipid production of 10.6 g/L. Accumulation of intracellular lipids up to 69% w/w, with a glucose-to-lipid conversion efficiency of 0.27 g/g.	TRL 4	Parisis 2023
Debaryomyces hansenii	100 mL. Low N content medium. Marine strain.	After 24h of cultivation, due to nitrogen exhaustion, lipids started to accumulate. after 72h, the biomass reached a lipid content of 20.7% (dw).	TRL 4	Donzella 2021
Cutaneotrichosporon oleaginosus	1.3 L fed-batch bioreactor. Industrial paper mill lignocellulosic hydrolysate. pH 6.5, at 28 °C and with a dissolved oxygen content of 50%	Employing pentose-rich LCH as a carbon source instead of glucose significantly improved both biomass formation and lipid titre, reaching 55.73 \pm 5.20 g/L and 42.1 \pm 1.7 g/L (75.5% lipid per biomass), respectively.	TRL 4	Rerop 2023
Ashbya gossypii (genetically modified)	2 L batch reactor. Non- Detoxified Lignocellulosic Biomass Hydrolysate medium (84.05 g/L glucose and 8.65 g/L xylose)	Lipid titre 1,42 g/L, Lipid content 11%	TRL 4	Francisco 2023
Lipomyces lipofer	Agitated culture on Pomegranate residues hydrolysate, at pH 6.5	X max of 2,1 g/L, Lipid accumulation of 7.0%, inhibition due to the presence of phenolic compounds. When the hydrolysate was diluted by 50%, Lipid accumulation increased to 13.5%, but X max reached only 1.0 g/L.	TRL 3	Dourou 2021
Pichia guilliermondii	14 L, batch. Crude glycerol plus corn steep liquor, 20 g/L. 120h at 28°C	24,47 g/L biomass with 52,09% lipid content. High C/N favoured lipid accumulation. Prevalence on linolenic, oleic and lignoceric acids production.	TRL4	Kumar 2027



To the best of our knowledge, no commercial plants utilizing single cell oils (SCOs) have been implemented yet. Nevertheless, the most advanced development we have identified involves a system capable of producing economically competitive biodiesel. This system utilizes a medium containing sugarcane juice and *Rhodosporidium toruloides*, operating within a 1500 L fed-batch reactor (Soccol et al. 2017). This achievement represents a significant milestone in the utilization of SCOs and underscores the promising future of these systems in the short to medium term.

It is evident that the majority of yeast strains are still in the stages of laboratory validation, typically ranging from TRLs 3 to 4. Despite their inherent potential, non-conventional strains require extensive further research and several years of development to attain commercial viability. Another significant factor hindering the advancement of TRLs is the need to optimize conditions for renewable feedstocks.

5.1.2 Strains for Isobutanol Production through Sugar Fermentation:

Isobutanol has emerged as a promising alternative to ethanol, the most widely produced biofuel globally (Aziz et al. 2023; Coimbra et al. 2023). While ethanol is derived from cane sugar or corn starch through fermentation, its suboptimal fuel properties, such as low energy density, high vapor pressure, and compatibility issues with existing fuel infrastructure, limit its classification as an advanced biofuel (Ni et al. 2023). In contrast, isobutanol and 1-butanol, categorized as higher alcohols, present notable advantages. These include superior energy density and lower vapor pressure compared to ethanol, positioning them as attractive candidates for advanced biofuel production and addressing the drawbacks associated with ethanol (Jawed et al. 2020; Zhang et al. 2019a).

Certain microbial hosts, as *S. cerevisiae*, *Pichia pastoris*, *Klebsiella pneumoniae*, *Magnusiomyces magnusii* and *Lactococcus lactis*, possess the natural ability to produce isobutanol (Wess, Brinek, and Boles 2019; Gu et al. 2017; Priyadharshini et al. 2015; Siripong et al. 2018). The intrinsic production of isobutanol by these microorganisms typically ranges from 0.01 to 0.44 g/L (Kurylenko et al. 2020; Priyadharshini et al. 2015). Recognizing the limited yields, researchers have implemented various metabolic engineering strategies to enhance the isobutanol biosynthetic pathways in these native host organisms, aiming to boost overall isobutanol synthesis (Kurylenko et al. 2020). These strategies encompass genetic modifications and pathway optimizations, reflecting concerted efforts to improve the efficiency of isobutanol production. Native hosts, while extensively studied, face limitations in achieving high isobutanol yields. The TRL for these strains is considerably progressing due to years of research (TRL 3). However, their short-term feasibility is hampered by relatively low isobutanol production levels and a reliance on expensive sugars such as glucose and lactose, as stated in Table 5-2.

Wess, Brinek, and Boles (2019) aimed to enhance isobutanol production in *S. cerevisiae* by overexpressing key endogenous enzymes in the valine synthesis pathway and optimizing metabolic flux. Overexpression of acetolactate synthase (IIv2), acetohydroxyacid reductoisomerase (IIv5), and dihydroxy-acid dehydratase (IIv3), along with disrupting the first step of mitochondrial valine synthesis, led to a significant 22-fold increase in isobutanol production. Subsequent deletions of essential genes in competing pathways further improved metabolic flux, resulting in titres of up to 2.09 g/L, with a remarkable yield of 59.55 mg/g glucose. However, the study acknowledges limitations in the intrinsic capacity of the isobutanol synthesis pathway, indicating areas for further exploration and improvement. A. Zhang et al. (2019a), on the other side, used adaptive laboratory evolution (ALE), to obtain a *S. cerevisiae* strain (EMS39) more tolerant to higher isobutanol and glucose concentrations. Over-expressing key genes this author obtained a strain with 32.4% higher isobutanol productivity compared to the control. Isobutanol titres reached 4.20 g/L, demonstrating the success of the approach. Whole genome resequencing and transcriptomic analysis revealed crucial mutations and transcriptional perturbations, providing insights into the molecular mechanisms influencing isobutanol tolerance and production in *S. cerevisiae*.



Table 5-2: Summary of isobutanol producing hosts.

			Con		Isobutanol		
	Species	Substract	Overexpressed genes	Knockouts	Promoters	titre	Reference
	Saccharomyces cerevisiae	Glucose 20 g/L	ILV2, ILV5, ILV3	ΔΙLV2, ΔΒDH1, ΔΒDH2, ΔLEU4, ΔLEU9, ΔECM31, ΔILV1, ΔADH1, ΔGPD1, ΔGPD2, ΔALD6	Pfba1, Ppfk1	2.09 g/L	Wess, Brinek, and Boles 2019
	Saccharomyces cerevisiae	Glucose 20 g/L	alsS, ILV2, ILV5, ILV3	ΔBAT1, ΔALD6	Padh1, Pcup1, Ptdh3	0.2632 g/L	Park and Hahn 2019
	Saccharomyces cerevisiae	Glucose 40 g/L	ILV3, ILV2, ILV5, ARO10			4.20 g/L	Zhang et al. 2019a
	Saccharomyces cerevisiae	Xylose 20 g/L	XI, XR, XDH, ILVs, KDC, ADH	ΔΒΑΤΊ, ΔALD6, ΔΡΗΟΊ3	Ptdh3, PTEF1, PPGK1, PADH1	3.10 g/L	Zhang et al. 2019b
il host	Candida sp.	Glucose, Valine				0.96 g/L	Lakshmi et al. 2021
Natro	Klebsiella pneumoniae	Glucose	Kivd	ΔbudA, ΔldhA	Pbud	3.19 g/L	Gu et al. 2017
	Lactococcus lactis	Lactose				0.01 g/L	Priyadhars hini et al. 2015
	Magnusiomyces magnusii	Glucose				0.44 g/L	Kurylenko et al. 2020
	Magnusiomyces magnusii	Glucose	ILV2		Ptac	0.62 g/L	Kurylenko et al. 2020
	Pichia pastoris	Glucose 20 g/L	Kivd, ADH7, ILV5, ILV3, ILV6, ILV2, ATF1		Pgap	2.22 g/L	Siripong et al. 2018
	Bacillus subtilis	Glucose 10 g/L	alsS, ilvC, ilvD, kivD, adh2, zwf, pntBA, udhA	Δldh, ΔpdhC and Δpgi	P43	6.12 g/L	Qi et al. 2014
	Clostridial fusants	Glucose 10 g/L				15 g/L	Roy and Dahman 2023
ost	Clostridium cellulolyticum	Cellobiose 5 g/L	kivd, alsS, ilvC, ilvD, yqhD		P <i>fdx</i>	0.660 g/L	Higashide et al. 2011
ural h	Clostridium	Frutose 40	kivd, adh, kor,	ilvE inactivation	Ppta–ack	2.4 mM	Weitz et
Non-natı	glutamicum	Glucose	Aant ilvBN, ilvCTM, ilvD, kivd, adhA, gapA, pgk, tpi, pfkA, pgi, zwf, edd, eda	ΔpckA, Δppc, ΔldhA, ΔilvE	Plac(AA), Plac(GA)	20.8 g/L	ai. 2021 (Hasegawa et al. 2020)
	Escherichia coli	Glucose, M9Y	alsS, ilvC, ilvD, kivd, LeuDH, yqhD	ΔaraBAD, ΔmcrA, ΔendA, ΔrecA, ΔmcrBC-hsdSMR- mrr	PLlacO1	5.76 g/L	Wang et al. 2020



Geobacillus	Glucose	Kivd, alsS, ilvC,		Pldh	3.3 g/L	Lin et al.
thermoglucosidasius		ilvD, adhA			-	2014
Ralstonia eutropha	Fructose	phaJ, sbm1, phaA, phaB1, ter, bldh, yqhD		PCAT, PphaC	32 mg/L	Black et al. 2018a
Synechococcus elongatus	CO ₂	alsS, ilvC, ilvD, kivd, yqhD	∆glgC	Ptrc, PLlacO1	550 mg/L	Li, Shen, and Liao 2014c
Synechocystis sp.	CO ₂	ilvBN, ilvC, ilvD, kdc, adh		PcpcG2	238 mg/L	Kobayashi et al. 2022a

In another study, (Y. Zhang et al. 2019b) optimized isobutanol production in *S. cerevisiae* by integrating the xylose isomerase pathway with the mitochondrial isobutanol biosynthetic pathway. Strategic deletions of PHO13, ALD6, and BAT1 were performed to enhance xylose assimilation and isobutanol production. Additional copies of the mitochondrial isobutanol pathway were introduced, resulting in a remarkable achievement – the strain produced a record 3.10 ± 0.18 g/L of isobutanol and 0.91 ± 0.02 g/L of 2-MbOH from xylose. This represents the highest reported isobutanol titre and yield from xylose, showcasing a significant advancement (28- and 9.5-fold higher than previous reports). The study also demonstrated the first-ever production of 2-MbOH from xylose.

In the long-term, the feasibility of using natural hosts for isobutanol production is moderate, with potential improvements through continued research, but fundamental limitations as the ones mentioned above may persist.

Progressing beyond native hosts, strains engineered from non-native microbial hosts like *E. coli* and *C. glutamicum* exhibit a moderate TRL status, with substantial progress in genetic engineering. The genetic engineering advancements in these strains have led to improved isobutanol production. In the short term, their feasibility is promising, although challenges like solvent toxicity need addressing. For instance, *E. coli* strains modified through transcription factor engineering have shown enhanced growth rates and tolerance to isobutanol. Through ongoing research, improvements in strain engineering may address these current limitations, with high potential for advancements in the long term.

Strains like *Clostridial fusants* have been developed through mutation breeding, showcasing a TRL advancement but falling short in terms of commercial viability. Roy and Dahman (2023) aimed to enhance the resistance of fused bacterial strains to biobutanol toxicity through UV radiation and EMS-induced mutations, reaching 15 g/L, surpassing the pre-mutation production of 13.8 g/L in the fused strain. A noteworthy 5.8% increase in biobutanol production yield was observed in the fused strains, suggesting their improved ability to handle biobutanol toxicity and achieve higher biobutanol yields. The study also highlighted an enhancement in oxygen tolerance for the mutated anaerobic *Clostridial fusant*, demonstrating the feasibility of mutation in fused strains and the utility of these techniques in identifying robust and stable fused bacterial strains.

Various innovative approaches involve exploring cellulolytic, photosynthetic, chemolithoautotrophic, and solvent-tolerant microorganisms. While these organisms are at different developmental stages in terms of TRL, their short-term feasibility is limited due to significant challenges that demand extensive research. Breakthroughs in these areas are crucial for long-term feasibility.

Innovations in modifying transcription factors (e.g., CRP), overexpressing resistance genes, and leveraging mutagenesis techniques have led to breakthroughs in <u>enhancing microbial tolerance to</u> <u>isobutanol</u>, allowing for higher concentrations during fermentation. Notable examples include strains of *S. cerevisiae* and *E. coli* exhibiting improved tolerance and higher isobutanol production (A. Zhang et al. 2019a; Y. Zhang et al. 2019b).

The adoption of <u>biosensors for high-throughput screening of isobutanol-producing strains</u>, such as those using the transcription factor BmoR, represents a significant innovation (Yu et al. 2019b). This



approach accelerates strain selection and isolation, overcoming the limitations of traditional, labourintensive screening methods.

To address pathway imbalances, optimizing cellular processes, <u>breakthroughs in cofactor balancing</u> <u>and gene expression</u> have been achieved through the engineering of pathways involving NADPHdependent enzymes. Overexpression of genes encoding transhydrogenase-like shunts, as seen in *S. cerevisiae*, has resulted in improved NADPH supply and increased isobutanol titres (Matsuda et al. 2013).

The <u>exploration of non-native hosts</u> like *E. coli* and *C. glutamicum* represents an innovation in expanding the possibilities of isobutanol production. Breakthroughs include achieving higher isobutanol yields compared to native hosts, with ongoing research aimed at optimizing these strains for commercial-scale production.

<u>Investigating strains that efficiently utilize cost-effective carbon sources</u> also represents an innovative approach. Breakthroughs in this area could lead to more economically viable processes for isobutanol production. Ongoing studies aim to identify strains capable of utilizing a diverse range of renewable feedstocks, potentially reducing the cost of isobutanol production. Continued exploration of alternative carbon sources is crucial for sustainability and economic viability. Ongoing research reflects the dynamic nature of the field, aiming to overcome limitations and advance the TRL of isobutanol production strains.

5.1.3 Syngas production from gasification, gas conditioning and upgrading

Biofuel are gaining importance as an alternative source of liquid fuel, due to its renewability, chemical properties, and lower lifecycle emissions. Gasification technologies have been used for many years in applications such as heating and power generation. However, its application in drop-in biofuel production using Fischer-Tropsch (FT) synthesis has been based on feedstocks such as natural gas and, especially coal. So, changing to biobased feedstocks, brings a different set of challenges which have an important impact on the gasification technology selection and on the type of syngas cleaning and conditioning to be carried out. Fischer-Tropsch synthesis process is an already mature technology and, at a theoretical level, does not change with the substitution of fossil feedstocks by biobased ones. Nevertheless, different technologies are currently being investigated to allow small-scale FT (Shahabuddin et al. 2020)

The production of drop-in biofuels such as biojet is a complex process due to the characteristics of the biobased feedstock, including oxygen content and types and concentration of contaminants.

The quality of the syngas is critical for FT synthesis; therefore, syngas cleaning is a key step to ensure the liquid fuels production. The selection of gasification technology can reduce the extent of syngas cleaning required. However, this choice must be evaluated with the cost of the technology. For instance, plasma gasification produces the cleanest syngas and has feedstock flexible specifications, however, it has the highest operational cost. For the other hand, simpler and less costly gasification technologies produce a lower quality syngas which requires more extensive cleaning.

The syngas cleaning technologies don't have significant technical challenges but can be a very costly step if multiple cleaning technologies must be used. To find a balance in terms of quality and cost for each step is crucial for the successful implementation of the pathway drop-in biofuels (Susan van Dyk and Jack Saddler 2021).

From a technology perspective, any feedstock with properties or characteristics compatible to specific types of gasification technology can be implemented. Most of the lignocellulosic biomass feedstock (e.g. agricultural and forestry residues, woody crops and energy crops) can be processed in gasification-FT technologies (Ng, Farooq, and Yang 2021).



HEFA is the most commercially developed SAF. Other production routes still require further development mainly to reduction cost production. However, drop-in biofuel production from FT has potential advantages such as flexibility in terms of feedstock utilisation, because a wide range of feedstock can be applied such as, agricultural waste or MSW, with high GHG emission savings. The use of MSW in this route is of particular interest as it is used waste that would otherwise be placed in landfill with adverse environmental impact. Nevertheless, the availability of MSW as a feedstock for SAF, the increase in these wastes needed to meet the market demand remains a concern as it may not be sufficient (Ng, Farooq, and Yang 2021).

FT synthesis has been operating commercially, mainly using syngas based in natural gas (Shell - Bintulu in Malaysia and Qatar) and coal-based syngas (Sasol - South Africa). Most of the biomass gasification-FT technologies are still in the demonstration phase such as the BioTfueL project by Total (France), Velocys/Red Rock Biofuels (Austria and U.S. <u>https://www.altalto.com/technology/</u>) and SYNDIÈSE-BtS project by CEA/Air Liquide (France) (Ng, Farooq, and Yang 2021).

The FT fuels synthesis from bio-based gasification is just approaching commercialization (TRL 7-8), and the jet fuel produced through the FT route has been certified and can be blended up to 50% with fossil kerosene (Detsios et al. 2023).

The first commercial biomass gasification and FT plant, Sierra BioFuels Plant has been constructed by Fulcrum Bioenergy in Nevada, U.S. and started to operate in 2022 with a capacity of producing approximately 42 ML of renewable, low-carbon transportation fuels each year from approximately 175,000 tons of landfill waste (https://www.fulcrum-bioenergy.com/sierra-biofuels).

The company Velocys in collaboration with British Airways, is developing first commercial Fischer– Tropsch BtL plant in the UK in Immingham, to produce Sustainable Aviation Fuel (SAF) The project called Altalto Immingham is a waste-to-SAF projects and will be able to process municipal and commercial solid waste to produce 75 ML per year of sustainable fuels with net negative carbon emissions, saving 350,000 tonnes per year of CO2 and achieving a 150% reduction in greenhouse gases compared to conventional jet fuel (https://velocys.com/projects/altalto/).

5.2 Technological innovations

5.2.1 Non-conventional Oleaginous Yeasts for MO Production:

Currently, two main intracellular processes are recognized for lipid accumulation mechanisms, each distinct from the pathway responsible for producing acetyl-CoA. The "Ex novo" pathway is associated with the presence of hydrophobic feedstock, operating independently of nitrogen depletion. It manifests even during the exponential growth phase of fermentation and tends to result in lower triacylglycerides (TAGs) production. In contrast, the "De novo" process is more intricate, involving the breakdown of sugars and other hydrophilic substrates. This mechanism highly dependent on nitrogen scarcity and depletion. Accordingly, specific research efforts are currently underway to explore combined "ex novo" and "de novo" fermentation strategies, aiming to maximize lipid accumulation. (Huang et al. 2017)

It is also known the impact of different process conditions on process efficiency, namely:

- Feed substrate Studies have highlighted the importance of a high carbon-to-nitrogen (C/N) ratio for lipid synthesis. The optimal value is considerably dependent on the strain. Nitrogen absence causes the cells to shift their metabolism to lipid accumulation, rather than cell division. However, yeast requires initial nitrogen intake to promote protein synthesis and biomass initial growth. On the other hand, each feedstock contains specific inhibitors that hinder lipid accumulation.
- **Temperature** Although most oleaginous yeasts are mesophilic (~30 °C), the ideal operating temperature varies depending on the yeast strain. Nevertheless, considering energy costs,



ambient temperature is preferred. Under this assumption, further research can focus on identifying the most suitable yeast strains for ambient temperature ranges.

- pH Slightly acidic conditions are typically favoured in lipid accumulation research due to their impact on cell membrane surface properties, which affect the absorption process. However, some strains have shown better lipid accumulation results at higher pH levels, particularly when the feedstock is rich in volatile fatty acids (VFAs) such as acetic acid in fed-batch reactors.
- Aeration Efficient aeration has been shown to significantly improve biomass and lipid yields.
 However, excessive aeration has minimal impact on performance.
- Inoculation The impact of inoculation density on the process should also be considered.
 While initial biomass density does not significantly affect lipid yields, it greatly influences the growth rate.

Since the validation of non-conventional yeasts for SCOs production, some research has shifted from laboratory ideal culture mediums to real waste streams, which is a fundamental step for sustainable fuels production. This enables the identification of new interactions between yeast strains and numerous inhibitors.

For example, *D. hansenii* potential to valorise industrial waste streams, such as dairy and lignocellulosic materials have been reported. This yeast is tolerant to halophilic conditions, low pH values and resists several common inhibitors such as phenolic compounds. It also possesses an unusual ability to metabolize pentoses, such as xylose (Navarrete, Estrada, and Martínez 2022).

C. oleaginosus is another species suitable for culture medium based on lignocellulosic biomass and other organic acids. The lipid profile of this yeast is prevalent on unsaturated long-chain FAs, such as oleic and linoleic acids (Di Fidio et al. 2021b).

Fatty acids are a product from anaerobic digestion that can also be used to a great extent for lipid accumulation. Various species were tested (*Y. lipolytica, C. saturnus, C. curvatum* and *L. lipofer*) in a VFAs-rich digestate and the best result reached 36.9% lipids on dry biomass.

The usage of fed-batch reactors or the implementation of a two-stage process can further increase lipid accumulation. Slininger et al. (2016), reported that several strains can degrade nearly all available sugars present on lignocellulosic biomass hydrolysates, reaching lipid accumulations up 25-30 g/L (39-45% of the theoretical yield), by utilizing a two-stage process with an increase C/N ratio from 50-75 to 400-500.

Another factor that can push the development of an economically feasible technique is genetic engineering. As evidenced by an example provided in Table 5-1, *Y. lipolytica* was engineered to enhance oxidative stress tolerance, leading to a noteworthy lipid yield of 0,252 $g_{lipids}/g_{glucose}$, accounting for 93% of the maximum theoretical yield, according with the authors (Xu, Qiao, and Stephanopoulos 2017a). Several other examples can be found in the literature.

Additionally, non-oleaginous yeasts such *Ashbya gossypii* can be used as SCOs thanks to genetic modifications, as reported by Francisco et al. (2023).

To achieve economic viability, and in line with a circular bioeconomy approach, other strategies are being employed. Those strategies include the valorisation of by-products, with carotenoids, citric acid and proteins being examples, and co-cultivation with different yeasts or with microalgae, which can provide organic carbon or secondary metabolites to be taken by the yeast and produce O_2 while consuming CO_2 produced by yeast's metabolism. In fact, CO2 production may account up to 35-50% of the carbon consumed (Gallego-García et al. 2023).



5.2.2 Strains for Isobutanol Production:

Recent technological innovations in strains for isobutanol production have predominantly focused on enhancing fermentation processes and implementing genetic modifications. These advancements aim to overcome challenges related to feedback inhibition, solvent toxicity, and pathway imbalances, ultimately resulting in improved isobutanol yields.

One key area of innovation involves <u>genetic modifications</u> to address feedback inhibition of enzymes in the isobutanol synthetic pathway. Researchers have explored alternative or engineered enzymes, such as acetohydroxy acid synthase (AHAS) and acetolactate synthase (ALS), to mitigate feedback inhibition issues. For example, deleting the ILV6 gene in *S. cerevisiae* AHAS enzyme improved 2.2-fold isobutanol production, compared to the wild type, showcasing the potential of genetic modifications to alleviate feedback inhibition and enhance yields (Hammer and Avalos 2017).

Solvent toxicity poses a substantial challenge to isobutanol production during fermentation. Innovations in fermentation processes have focused on enhancing microbial tolerance to isobutanol. Strategies include the overexpression of resistance genes, artificial mutagenesis, and long-term adaptive laboratory evolution. For instance, *S. cerevisiae strains* evolved through ethyl methanesulfonate (EMS) mutagenesis and ALE demonstrated improved tolerance to higher concentrations of isobutanol (the evolved strain EMS39 performed better in higher concentrations of isobutanol (16 g/L) and glucose (100 g/L)), producing 30% more isobutanol than the control strain (A. Zhang et al. 2019a). Additionally, modifying transcription factors, such as cAMP protein (CRP) in *E. coli*, has proven to be effective to increase tolerance and growth rates (0.18 h⁻¹ compared to the control strain 0.05 h⁻¹) in the presence of 1.2% isobutanol (9.6 g/L, Chong et al. 2014).

Balancing metabolic pathways is another crucial aspect of strain improvement. Cofactor balancing and balanced gene expression have been implemented to optimize metabolic pathways for isobutanol production. Overexpression of genes encoding transhydrogenase-like shunts in *S. cerevisiae*, such as MDH2, PYC2, and MAE1, successfully adjusted the cofactor imbalance, leading to increased 1.84-fold isobutanol titres, compared with the strain BSW4 after 48 hours of fermentation (Matsuda el al. 2013). Similarly, balanced gene expression through promoter engineering, RBS engineering, and gene copy number control has demonstrated significant improvements. For instance, expressing the isobutanol synthetic pathway genes under different promoters resulted in varied isobutanol production (0.05 to 0.6 g/L) among recombinant strains of *C. thermocellum*, emphasizing the importance of balanced gene expression in optimizing yields (Lin et al. 2014a).

These technological innovations collectively contribute to the enhanced production of isobutanol by addressing key challenges in the fermentation process. Genetic modifications play a pivotal role in overcoming feedback inhibition, allowing for the use of alternative enzymes that are not hindered by end product regulation. Improved microbial tolerance achieved through fermentation process enhancements ensures the viability of strains in the presence of toxic concentrations of isobutanol. Additionally, pathway optimization through cofactor balancing and balanced gene expression maximizes the efficiency of isobutanol synthesis, resulting in higher yields.



$5 \cdot 2 \cdot 3$ Syngas production from gasification, gas conditioning and upgrading

Technical improvements in the gasification – FT route using biomass feedstocks are still needed and can mainly be divided into:

Gasification process

- Ability to use low-cost feedstocks such as municipal solid waste (MSW) without compromising the quality of the syngas.
- Development of intermediate processes, before gasification, to assure the homogeneity of the feedstock.
- Development of more efficient and less costly syngas cleaning methods (less steps).

FT synthesis

• Faster reaction rates (which are currently being pursued by companies such as Velocys).

Development of bifunctional catalysts that can produce a larger jet fraction (up to 70%) which can reduce the additional infrastructure normally required to either crack longer waxes or oligomerisation of shorter naphtha molecules into jet-range molecules (Susan van Dyk and Jack Saddler 2021).

5.3 Technological constraints

5.3.1 Non-conventional Oleaginous Yeasts for MO Production:

Numerous gaps remain to be filled given the diversity of oleaginous yeasts that are available and the variance of feedstock. However, several technological limitations have already been observed.

Since glucose medium is the sole source of carbon used in most research projects, substrate inhibition is one of the standard constraints that are mentioned. Therefore, batch reactors are unsuitable for producing SCOs. At the laboratory scale, operating sequential two-stage cultivation system, with focus on biomass growth, followed by a focus on lipid accumulation (Parisis et al. 2023; Slininger et al. 2016) might result in higher yields. However, in order to implement it in a large scale, at least two reactors must be used, which requires additional funding, space, and logistics. For this reason, adapting to fedbatch is typically more practical. To reduce costs, an open fermentation might be considered, although it adds contamination risks. These strategies enable an increase in C/N ratio.

With the use of waste and heterogenous feedstock, it is unavoidable the presence of growth inhibitors in the medium, which lower lipid titre. As response, research conducted with *Lipomyces lipofer* on Pomegranate residues hydrolysate tested the effect of diluting an inhibitor (phenolic compounds) presence by diluting the culture medium. The dilution of 50% with water increased lipid accumulation (w/w) from 7,0% to 13.5%, but at the cost of a reduction on biomass production, from 2,1 g/L to 1,0 g/L (Dourou et al. 2021). Feedstock can also lack the availability of other macronutrients, such as P and S, whose absence limits lipid accumulation.

Concerns about potential decreases in lipid yield and biomass production persist and must be addressed to ensure the economic viability of integrating oleaginous yeasts as bioenergy carriers. The costs of the feedstock and process must be reduced, and strains with improved inhibitory resistance and lipid production capacity should be developed, in which can be achieved through genetic engineering. Another option to reduce costs is related to byproduct valorisation, being carotenoids, proteins or other added value product synthetised by the yeast (Lopes da Silva et al. 2023a).

Regarding genetic modifications, the lack of genome sequences for many non-conventional oleaginous yeasts in public databases is a significant hurdle. This limitation has contributed to Yarrowia lipolytica's prominence in research studies focusing on this topic.



SCOs from non-conventional yeasts are a medium/long-term potential in-between process to produce SAFs from waste streams, as the accumulated lipids have similar compositions of those from the plants. However, numerous factors hinder their widespread adoption. In fact, SAF production is yet to be an economically competitive option in comparison with fossil jet fuel, which proves the need for further advancements in this field of study. There is no clear most promising strain. Additionally, there isn't a platform that employs a methodical approach to gather data from earlier studies.

5.3.2 Strains for Isobutanol Production:

Technological constraints in strains for isobutanol production present challenges that impede the scalability and metabolic efficiency of the process. Addressing these constraints is crucial for advancing the field and optimizing strains for industrial scale isobutanol production. One significant constraint lies in the metabolic limitations of microbial hosts used for isobutanol production. Pathway imbalances, cofactor availability, and competition for precursors within the cell often limit the efficiency of isobutanol synthesis. For instance, the imbalance between NADH and NADPH, essential cofactors for different reactions in the isobutanol pathway, poses a challenge. Further research should focus on developing more sophisticated strategies for cofactor balancing, potentially through the identification and incorporation of novel cofactor regeneration systems. Exploring alternative pathways or enzymes with improved catalytic activity can also address metabolic bottlenecks, enhancing the overall yield of isobutanol. The scalability of isobutanol production processes faces challenges related to fermentation scale-up and downstream processing. Microbial strains that perform well in laboratory-scale fermentations may exhibit different behaviors at larger scales. Variability in environmental conditions, substrate utilization efficiency, and productivities can impact the consistency and reliability of isobutanol production. To ensure cost-effectiveness and minimal environmental impact it is essential to develop optimized and robust scalable fermentation technologies and efficient downstream processes. These include continuous fermentation systems, in situ product removal techniques, and advanced separation technologies to enhance product recovery and purification.

In terms of further research or development, several avenues can be explored to overcome these constraints. Firstly, synthetic biology tools can be leveraged for enhanced metabolic engineering. Advanced technologies such as inducible promoters, precise CRISPR-based genome editing, and synthetic transcription factors can be employed to fine-tune metabolic pathways for improved isobutanol production. Strain optimization for robustness is another key area for exploration. Research strategies can be devised to enhance the robustness of microbial strains, making them more resilient to variations in fermentation conditions and substrate availability. This could involve the identification of stress-tolerant strains or the engineering of existing strains for improved adaptability. Adopting integrated systems biology approaches can provide a comprehensive understanding of cellular processes involved in isobutanol production. Systems-level analyses can offer insights into intricate regulatory networks, facilitating the identification of key targets for strain improvement. Exploring novel hosts and pathway engineering is also essential. This includes investigating non-traditional microbial hosts or unconventional pathways for isobutanol production. Extremophiles or synthetic consortia that can operate under diverse conditions may provide alternatives to overcome limitations observed in conventional hosts, for example.

The exploration of renewable feedstocks is also crucial. Investigating the utilization of a broader range of renewable feedstocks, such as lignocellulosic biomass and waste streams, can make isobutanol production more sustainable and economically viable. By addressing these technological constraints and investing in further research and development, the field of isobutanol production can overcome current limitations and move towards more efficient, scalable, and sustainable biofuel production.



$5 \cdot 3 \cdot 3$ Syngas production from gasification, gas conditioning and upgrading

The main challenges of producing sustainable aviation fuels from lignocellulosic biomass and residual wastes include: 1) low energy density of the feedstocks, 2) heterogeneity of feedstock in terms of chemical composition, physical properties and moisture content, 3) the complexity and high capital cost of the gasification, gas cleaning and FT process and 4) low carbon efficiency of the overall process. To achieve a higher SAF production from the FT process, further development of the technologies involved in this route are required to improve efficiency and reduce costs (Shahabuddin et al. 2020).

The major challenge of gasification-based biojet production is the high investment cost. Although operational costs can be relatively low, depend on the type of gasifiers used. The reduction of the gasification reactor costs is desirable, but it can compromise the quality of the syngas. Another costly component is the cleaning system of the raw syngas prior to Fischer-Tropsch synthesis. The syngas cleaning typically involves the multiple process steps needed to remove different contaminants. The feedstock variability and different levels of contaminants increase the complexity and cost of the process. Regarding the Fischer-Tropsch synthesis, it has been commercialized by the oil and gas sector for many decades being this knowledge more recently applied to biomass (Susan van Dyk and Jack Saddler 2021).

While the gasification of coal to syngas is a developed technology, there are challenges associated with the different nature of waste feeds in terms of syngas production, mainly significant variability in feedstock properties can be expected. Although gasification technology can work with some variation in feed material composition, gasifiers are designed for either a solid or a liquid feed, and when a solid feed is employed, particle size as ash content must be considered. Also, the need for economy of scale to achieve an economically will implies the use of waste materials obtained over a geographically wide region which will be challenging in terms of cost and energy requirements for transport of low energy density waste feed materials. So, when dealing with diverse sources of waste, ranging from logging residues to municipal sewage sludge, an intermediate step before gasification can be considered, because it is technically difficult to ensure that there will be sufficient homogeneity in terms of feed composition, including particle size distribution, to implement a robust process using gasification of solid mixed feed materials (Montoya Sánchez et al. 2022).

An advantage of this route is that the properties of the synthetic crude obtained via FT synthesis are independent of the nature of the raw feedstock for gasification due to the intermediate syngas production step. Therefore, SAF production from waste-based feedstocks via gasification and FT synthesis can be predicted (Montoya Sánchez et al. 2022).

Fuels derived from biogenic wastes and residues (lignocellulosic feedstock) via the FT routes normally provide reductions of GHG emissions. The wide feedstock flexibility of these technologies and the technological advances expected can limit their production costs and contribute for the SAF demand that is expected to arise due to HEFA constraints (Detsios et al. 2023).

In summary, conceptually the use of waste feed materials to produce SAF is straightforward and many suggestions to do so can be found the literature. The main concerns are linked to the high scale that must be achieved for SAF to meaningfully contribute to aviation fuel, and with the challenge of developing an economical process for dealing with the diverse nature of distributed low energy density waste (Montoya Sánchez et al. 2022).



6 Conclusions

Important feedstocks for SAF from the agriculture sector are vegetable oils (i.e. rapeseed, carinata, camelina) and non-food cellulosic species (i.e. biomass sorghum, legume cover crops, switchgrass, miscanthus) that can be grown as dedicated energy crops and/or as intermediate crops in several innovative cropping schemes with low ILUC risks and at the same time able to improve soil health and biodiversity. Vegetable oils are suitable feedstocks for SAF through HEFA technologies, however, they present concerns over the species' low yields, and therefore high cost and availability. On the other hand, lignocellulosic feedstocks are cheaper and with considerably higher biomass yield production potential, but still need to resolve issues related to the complicated and costly conversion process (i.e. FT synthesis pathways). Despite their potential benefits, innovative multiple cropping systems with these species are still underutilized as SAF feedstock source. To comply with the increasing demands of SAF feedstocks from the agricultural sector, potential solutions should go, among others, through the development and establishment of long-term innovative low ILUC risk cropping systems.

Another significant feedstock source for producing SAF is the residues from crops and waste streams. The RED II Annex IX A (as only advanced biofuels are addressed in the ReFuelEU Aviation) determines the biomass sources that can be considered as potential feedstock. Straw is the most abundant among those sources and the easiest to collect. To secure large-scale straw supply of satisfactory quality at reasonable prices, straw handling must be carried out as efficiently as possible. Currently, there is a significant amount of expertise in dealing with straw management, and effective logistics and supply chain systems have been established. As a result, delivering straw is not a problem, provided that the required quantities have been secured.

Another very important source for SAF production feedstock is the lignocellulosic residues from fruit trees, which are basically produced from pruning. Collecting wood from pruning presents logistical challenges due to several factors: (i) its dispersion across territories, (ii) the size and layout of plantations, and (iii) the relatively low biomass production per hectare compared to forestry wood. However, this waste is an excellent biomass source. Agricultural pruning has the potential to be used extensively for energy production, but its utilization is limited due to several constraints such as high costs and lack of technology.

Lignocellulosic residues can be produced from forestry as well. Most of the residues coming out from the indirect use of forest wood can be used for biofuel production. The process of recovering waste wood from various sources is relatively straightforward. The timber processing industry usually collects every waste generated during their operations, which is then utilized by the same industry for energy production. Additionally, wood from municipal waste is either recovered on-site through recycling schemes or at landfills through mechanical waste separation plants. The tricky case is to recover wood residues that are left in the forest during the cutting process.

Dried sewage sludge can be also used as a potential feedstock for the production of SAF through thermochemical pathways. Sewage sludge or residues from industrial processes are wastewaters with high organic content. Finally, municipal solid waste can be a potential source of feedstock for the production of SAF. Carbon-based waste such as product packaging, grass clippings, furniture, clothing, bottles, food scraps and newspapers can be properly processed and converted into SAF. There is an increasing trend in MSW generation, which justifies the importance of this source of potential SAF feedstock. Another fact that supports the case of MSW is that in recent years an increasing amount of MSW are properly treated and most of the useful fraction of them are recovered, leaving only a small portion for landfilling.

Technical innovations regarding biomass residues that are suitable for SAF production focus on feedstock collection, biomass handling and processing equipment optimization, and strategies for mobilizing biomass residues effectively and building integrated biomass supply chains.

The technological constraints for biomass residues valorisation in SAF production processes are:



- High variability of the types of residues (straw, tree pruning, forest residues) with different fuel characteristics, requiring different harvesting/collection equipment
- Dispersed geographic allocation leads to high transportation costs
- Location of the feedstock production site and its proximity to conversion plants --> transportation costs
- Seasonal availability and limited harvesting window requiring time-efficient biomass collection systems
- High feedstock prices and limited availability and scalability of sustainable feedstock

Besides feedstock, the energy carriers, an intermediate between raw feedstock and SAF, are very important for the further development of the SAF market. Three key bioenergy carriers were investigated: Non-conventional oleaginous yeasts for optimizing microbial oil, isobutanol production strains, and syngas production.

Oleaginous organisms possess the ability to store a minimum of 20% of their dried biomass as internal lipids. The review of the research TRL on different non-conventional oleaginous yeast species showed that are typically range from TRLs 3 to 4. Currently, two main intracellular processes are recognized for lipid accumulation mechanisms. The "Ex novo" pathway is associated with the presence of hydrophobic feedstock, operating independently of nitrogen depletion. The "De novo" process is more intricate, involving the breakdown of sugars and other hydrophilic substrates. This mechanism is highly dependent on nitrogen scarcity and depletion. Despite their inherent potential, non-conventional oleaginous yeast strains require extensive research and several years of development to attain commercial viability. Another significant factor hindering the advancement of TRLs is the need to optimize conditions for renewable feedstocks. Substrate inhibition is one of the standard constraints that are mentioned. With the use of waste and heterogeneous feedstock, it is unavoidable the presence of growth inhibitors in the medium, which lower lipid titre. Finally, the lack of genome sequences for many non-conventional oleaginous yeasts in public databases is a significant hurdle.

Isobutanol has emerged as a promising alternative to ethanol, the most widely produced biofuel globally. Recognizing the limited yields of its production, researchers have implemented various metabolic engineering strategies to enhance the isobutanol biosynthetic pathways in these native host organisms, aiming to boost overall isobutanol synthesis. Ongoing studies aim to identify strains capable of utilizing a diverse range of renewable feedstocks, potentially reducing the cost of isobutanol production. Continued exploration of alternative carbon sources is crucial for sustainability and economic viability. Ongoing research reflects the dynamic nature of the field, aiming to overcome limitations and advance the TRL of isobutanol production strains, which is now at Level 3.

Gasification technologies have been used for many years in applications such as heating and power generation. However, its application in drop-in biofuel production using Fischer-Tropsch (FT) synthesis has been based on biomass feedstocks, brings a different set of challenges which have an important impact on the gasification technology selection and on the type of syngas cleaning and conditioning to be carried out. Fischer-Tropsch synthesis process is an already mature technology and, at a theoretical level, does not change with the substitution of fossil feedstocks by biobased ones. Nevertheless, different technologies are currently being investigated to allow small-scale FT. FT synthesis has been operating commercially, mainly using syngas based in natural gas. Most of the biomass gasification-FT technologies are still in the demonstration phase. The FT fuels synthesis from bio-based gasification is just approaching commercialization (TRL 7-8), and the jet fuel produced through the FT route has been certified and can be blended up to 50% with fossil kerosene. The first commercial biomass gasification and FT plant, Sierra BioFuels Plant has been constructed by Fulcrum Bioenergy in Nevada, U.S. and started to operate in 2022 with a capacity of producing approximately 42 ML of renewable, low-carbon transportation fuels each year from approximately 175,000 tons of landfill waste. Velocys in collaboration with British Airways, is developing first commercial Fischer–Tropsch BtL plant in the UK in Immingham, to produce 75 ML per year Sustainable Aviation Fuel (SAF) from municipal and commercial solid waste



7 References

Abideen, Z., Ansari, R., Gul, B. and Khan, A. 2012. "The Place of Halophytes in Pakistan's Biofuel Industry." *Biofuels* 3 (2): 211–20.

Air Transport Action Group (2023). Beginners guide to SAF. Available at: <u>https://aviationbenefits.org/media/168027/atag-beginners-guide-to-saf-edition-2023.pdf</u>

Alhotan, RA., Wang, RL., Holser, RA., and Pesti, GM. 2017. "Nutritive Value and the Maximum Inclusion Level of Pennycress Meal for Broiler Chickens." *Poultry Science* 96 (7): 2281–93.

Aliano-González, MJ, Gabaston, J., Ortiz-Somovilla, V. and Cantos-Villar, E. 2022. "Wood Waste from Fruit Trees: Biomolecules and Their Applications in Agri-Food Industry." *Biomolecules* 12 (2): 238.

Angelini, L., Moscheni, E., Colonna, G., Belloni, P. and Bonari, E. 1997. "Variation in Agronomic Characteristics and Seed Oil Composition of New Oilseed Crops in Central Italy." *Industrial Crops and Products* 6 (3–4): 313–23.

Aziz, T., Shah, Z., Sarwar, A, Ullah, N., Khan, A., Sameeh, M., Haiying, C. and Lin L. 2023. "Production of Bioethanol from Pretreated Rice Straw, an Integrated and Mediated Upstream Fermentation Process." *Biomass Conversion and Biorefinery*, May. <u>https://doi.org/10.1007/s13399-023-04283-w</u>.

Baldino, C., Pavlenko, N., Searle, S. and Christensen, A. 2018. The potential for low-carbon renewable methane in heating, power, and transport in the European Union. Retrieved from the International Council on Clean Transportation: https://theicct. org/sites/default/files/publications/Renewable_Gas_EU-28_20181016. pdf.

Barla, F. G, and Kumar, S. 2019. "Tobacco Biomass as a Source of Advanced Biofuels." *Biofuels* 10 (3): 335–46.

Barnes, D, Baldwin, B and Braasch, D. 2009. "Degradation of Ricin in Castor Seed Meal by Temperature and Chemical Treatment." *Industrial Crops and Products* 29 (2–3): 509–15.

Becken, S., Mackey, B. and Lee, D. 2023. "Implications of Preferential Access to Land and Clean Energy for Sustainable Aviation Fuels." *Science of The Total Environment* 886:163883.

Berti, M., Gesch, R., Johnson, B., Ji, Y., Seames, W. and Aponte, A. 2015. "Double-and Relay-Cropping of Energy Crops in the Northern Great Plains, USA." *Industrial Crops and Products* 75:26–34.

Black, W., Zhang, L., Kamoku, C., Liao, J. and Li, H. 2018a. "Rearrangement of Coenzyme A-Acylated Carbon Chain Enables Synthesis of Isobutanol via a Novel Pathway in Ralstonia Eutropha." ACS Synthetic Biology 7 (3): 794–800.

Carr, P. M. (1993). Potential of fanweed and other weeds as novel industrial oilseed crops. J. Janick, and J. E. Simon (eds). New crops, p. 384–388

Coimbra, L., Malan, K., Fagúndez, A., Guigou, M., Lareo, C., Fernández, B., Pratto, M. and Batista S. 2023. "Fermentation of D-Xylose to Ethanol by Saccharomyces Cerevisiae CAT-1 Recombinant Strains." *BioEnergy Research* 16 (2): 1001–12. https://doi.org/10.1007/s12155-022-10514-1.

Commission, European, and Directorate-General for Energy. 2024. Union Bioenergy Sustainability Report – Study to Support Reporting under Article 35 of Regulation (EU) 2018/1999 – Final Report. Publications Office of the European Union. <u>https://doi.org/doi/10.2833/527508</u>

Czarnik, M., Jarecki, W. and Bobrecka-Jamro, D. 2018. "Reaction of Winter Varieties of False Flax (Camelina Sativa (L.) Crantz) to the Varied Sowing Time." *Journal of Central European Agriculture* 19 (3): 571–86.

Del Gatto, A., Melilli, M., Raccuia, S., Pieri, S., Mangoni, L., Pacifico, D., Signor, M., Duca, D., Pedretti, E. and Mengarelli, C. 2015. "A Comparative Study of Oilseed Crops (Brassica Napus L. Subsp. Oleifera



and Brassica Carinata A. Braun) in the Biodiesel Production Chain and Their Adaptability to Different Italian Areas." *Industrial Crops and Products* 75:98–107.

Detsios, N., Theodoraki, S., Maragoudaki, L., Atsonios, K., Grammelis, P. and Orfanoudakis, N. 2023. "Recent Advances on Alternative Aviation Fuels/Pathways: A Critical Review." *Energies* 16 (4). https://doi.org/10.3390/en16041904.

Di Fidio, N., Minonne, F., Antonetti, C. and Raspolli Galletti A.M. 2021a. "Cutaneotrichosporon Oleaginosus: A Versatile Whole-Cell Biocatalyst for the Production of Single-Cell Oil from Agro-Industrial Wastes." *Catalysts* 11 (11). https://doi.org/10.3390/catal11111291.

Diamantopoulou, P., Sarris, Δ., Tchakouteu, S.S., Xenopoulos, E., and S. Papanikolaou. 2023. "Growth Response of Non-Conventional Yeasts on Sugar-Rich Media: Part 1: High Production of Lipid by Lipomyces Starkeyi and Citric Acid by Yarrowia Lipolytica." *Microorganisms* 11 (7): 1863.

Donzella, S., Capusoni, C., Pellegrino, L., Compagno, C. Bioprocesses with Reduced Ecological Footprint by Marine Debaryomyces hansenii Strain for Potential Applications in Circular Economy. J. Fungi 2021, 7, 1028. https://doi.org/10.3390/jof7121028.

Dourou, M., Christina N. Economou, Lida Aggeli, Miroslav Janák, Gabriela Valdés, Nefeli Elezi, Dimitrios Kakavas, et al. 2021. "Bioconversion of Pomegranate Residues into Biofuels and Bioactive Lipids." *Journal of Cleaner Production* 323:129193. https://doi.org/10.1016/j.jclepro.2021.129193.

EPRS | European Parliamentary Research Service (2020). Sustainable aviation fuels.

Fan, J., Shonnard, D. R., Kalnes, T. N., Johnsen, P. B., & Rao, S. 2013. A life cycle assessment of pennycress (Thlaspi arvense L.) -derived jet fuel and diesel. Biomass and Bioenergy, 55, 87–100. https://doi.org/10.1016/j.biombioe.2012.12.040

FAO (2012). Environment and Natural Resources Working Paper No.49 – FAO, Rome, 2012.

Francisco, M., Aguiar, T.Q., Abreu, G., Marques, S., Gírio, F. and Domingues, L. 2023. "Single-Cell Oil Production by Engineered Ashbya Gossypii from Non-Detoxified Lignocellulosic Biomass Hydrolysate." *Fermentation* 9 (9). https://doi.org/10.3390/fermentation9090791.

Folic M. 2023. Feedstuck Resolving short- and long-term feedstock issues in SAF production via hydroprocessing. TOPSOE.

Forero, C. 2005. "Biodiesel from Castor Oil: A Promising Fuel for Cold Weather." RE&PQJ 3 (1).

Gallego-García, M., Susmozas, A., Negro, M. J. and Moreno, A. 2023. "Challenges and Prospects of Yeast-Based Microbial Oil Production within a Biorefinery Concept." *Microbial Cell Factories* 22 (1): 246. https://doi.org/10.1186/s12934-023-02254-4.

Gesch, RW, Mohammed, YA., Walia, MK., Hulke, BS. and Anderson, JV. 2022. "Double-Cropping Oilseed Sunflower after Winter Camelina." *Industrial Crops and Products* 181:114811.

Glenn, E. P., Brown, J. and Blumwald, E. 1999. "Salt Tolerance and Crop Potential of Halophytes." *Critical Reviews in Plant Sciences* 18 (2): 227–55.

Glenn, E. P., O'Leary, J. W., Watson, C., Thompson, L, nd Robert O Kuehl. 1991. "Salicornia Bigelovii Torr.: An Oilseed Halophyte for Seawater Irrigation." *Science* 251 (4997): 1065–67.

Gopinathan, M. and Sudhakaran, R. 2009. Biofuels: Opportunities and Challenges in India." *In Vitro Cellular & Developmental Biology-Plant* 45:350–71. http://dx.doi.org/10.1007/s11627-009-9217-7

Gorgec, A., Insel, G., Yagci, N., Dogru, M., Erdinçler, A., Sanin, D, Filibeli, A., Keskinler, B. and Cokgor, E. 2016. "Comparison of Energy Efficiencies for Advanced Anaerobic Digestion, Incineration, and Gasification Processes in Municipal Sludge Management." *Journal of Residuals Science & Technology* 13 (January):57–64. https://doi.org/10.12783/issn.1544-8053/13/1/8.

Grisan, S., Polizzotto, R., Raiola, P. et al. Alternative use of tobacco as a sustainable crop for seed oil, biofuel, and biomass. Agron. Sustain. Dev. 36, 55 (2016). <u>https://doi.org/10.1007/s13593-016-0395-5</u>.



Gu, J., Zhou, J., Zhang, Z., Ho Kim, C., Jiang, B., Shi, J. and Hao, J. 2017. "Isobutanol and 2-Ketoisovalerate Production by Klebsiella Pneumoniae via a Native Pathway." *Metabolic Engineering* 43:71–84.

Gul, B., Abideen, Z., Ansari, R. and Khan, A. 2013. "Halophytic Biofuels Revisited." *Biofuels* 4 (6): 575–77.

Hammer, S., and Avalos, J. 2017. "Uncovering the Role of Branched-Chain Amino Acid Transaminases in Saccharomyces Cerevisiae Isobutanol Biosynthesis." *Metabolic Engineering* 44:302–12. https://doi.org/10.1016/j.ymben.2017.10.001.

Hasegawa, S., Jojima, T., Suda, M. and Inui, M. 2020. "Isobutanol Production in Corynebacterium Glutamicum: Suppressed Succinate by-Production by pckA Inactivation and Enhanced Productivity via the Entner–Doudoroff Pathway." *Metabolic Engineering* 59:24–35.

Higashide, W., Li, Y., Yang, Y. and Liao, J. 2011. "Metabolic Engineering of Clostridium Cellulolyticum for Production of Isobutanol from Cellulose." *Applied and Environmental Microbiology* 77 (8): 2727–33.

Hossain, Z, Johnson, E., Blackshaw, R., Liu, K., Kapiniak, A., Gampe, C., Molnar, L., Luan, L., Poppy, L. and Gan, Y. 2018. "Agronomic Responses of Brassica Carinata to Herbicide, Seeding Rate, and Nitrogen on the Northern Great Plains." *Crop Science* 58 (6): 2633–43.

Huang, C, Luo, M., Chen, X., Qi, G., Xiong, L., Lin, X., Wang, C., Li, H. and Chen, X. 2017. "Combined 'de Novo' and 'Ex Novo' Lipid Fermentation in a Mix-Medium of Corncob Acid Hydrolysate and Soybean Oil by Trichosporon Dermatis." *Biotechnology for Biofuels* 10 (1): 147. <u>https://doi.org/10.1186/s13068-017-0835-8</u>.

IEA | Intenational Energy Agency (2022). Renewables 2022. Analysis and forecast to 2027.

Jankowski, K., Sokolski, M. and Kordan, B. 2019. "Camelina: Yield and Quality Response to Nitrogen and Sulfur Fertilization in Poland." *Industrial Crops and Products* 141:111776.

Jawed, K., Abdelaal, A., Koffas, M. and Yazdani, S. 2020. "Improved Butanol Production Using FASII Pathway in E. Coli." *ACS Synthetic Biology* 9 (9): 2390–98. https://doi.org/10.1021/acssynbio.0c00154.

Jordan, N., Dorn, K., Runck, B., Ewing, P., Williams, A., Anderson, KA., Felice, L., Haralson, K., Goplen, J. and Altendorf, K. 2016. "Sustainable Commercialization of New Crops for the Agricultural Bioeconomy." *Elementa* 4:000081.

Kaltschmitt, M., and Neuling, U. 2017. *Biokerosene: Status and Prospects*. Springer.

Kobayashi, S., Atsumi, S., Ikebukuro, K., Sode, K. and Asano R. 2022a. "Light-Induced Production of Isobutanol and 3-Methyl-1-Butanol by Metabolically Engineered Cyanobacteria." *Microbial Cell Factories* 21 (1): 7.

Kumar, A., Singh, P., Singh, DP., Singh, H., and Sharma, HC. 1984. "Differences in Osmoregulation in Brassica Species." *Annals of Botany* 54 (4): 537–42. https://doi.org/10.1093/oxfordjournals. aob.a086824

Kumar, N., Sonthalia, A., Pali, H. and Sidharth. 2020. "Next-Generation Biofuels—Opportunities and Challenges." In *Innovations in Sustainable Energy and Cleaner Environment*, edited by Ashwani K. Gupta, Ashoke De, Suresh K. Aggarwal, Abhijit Kushari, and Akshai Runchal, 171–91. Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-9012-8_8.

Kumar, S., Seepaul, R., Mulvaney, M., Colvin, B., George, S., Marois, J., Bennett, R., Leon, R., Wright, D. and Small, I. 2020. "Brassica Carinata Genotypes Demonstrate Potential as a Winter Biofuel Crop in South East United States." *Industrial Crops and Products* 150:112353.

Kumar, R., Dhanarajan, G., Bhaumik, M., Chopraab J and Sen, R. 2017. Performance evaluation of a yeast biorefinery as a sustainable model for co-production of biomass, bioemulsifier, lipid, biodiesel



and animal-feed components using inexpensive raw materials. Sustainable Energy Fuels, 2017, 1, 923 DOI: 10.1039/c7se00010c.

Kurylenko, O., Ruchala, J., Dmytruk, K., Abbas, C. and Sibirny, A. 2020. "Multinuclear Yeast Magnusiomyces (Dipodascus, Endomyces) Magnusii Is a Promising Isobutanol Producer." *Biotechnology Journal* 15 (7): 1900490.

Lakshmi, N., Binoop, M., Salini, C., Vivek, N., Sindhu, R., Pandey A., and Binod, P. 2021. "Isobutanol Production by Candida Glabrata–A Potential Organism for Future Fuel Demands." *Fuel* 306:121634.

Li, X., Shen, C. and Liao, J. 2014a. "Isobutanol Production as an Alternative Metabolic Sink to Rescue the Growth Deficiency of the Glycogen Mutant of Synechococcus Elongatus PCC 7942." *Photosynthesis Research* 120:301–10.

Lin, P., Rabe, K., Takasumi, J., Kadisch, M., Arnold, F. and Liao, J. 2014a. "Isobutanol Production at Elevated Temperatures in Thermophilic Geobacillus Thermoglucosidasius." *Metabolic Engineering* 24:1–8.

Liu, S., Zhu, Q., Guan, Q., He, L. and Li, W. 2015. "Bio-Aviation Fuel Production from Hydroprocessing Castor Oil Promoted by the Nickel-Based Bifunctional Catalysts." *Bioresource Technology* 183:93–100.

Lonard, RI., Judd, FW., Stalter, R. 2012. The biological flora of coastal dunes and wetlands: Salicornia bigelovii J. Torrey. J Coast Res 28(3):719–725. doi:10.2112/JCOASTRES-D-11-00125.1

Lopes da Silva, T., Fontes, A., Reis, A., Siva, C. and Gírio, F. 2023a. "Oleaginous Yeast Biorefinery: Feedstocks, Processes, Techniques, Bioproducts." *Fermentation* 9 (12): 1013.

Makkar, H.P.S. 2016. "State-of-the-Art on Detoxification of Jatropha Curcas Products Aimed for Use as Animal and Fish Feed: A Review." *Animal Feed Science and Technology* 222:87–99. <u>https://doi.org/10.1016/j.anifeedsci.2016.09.013</u>

Makkar, H.P.S., Kumar, V., Becker, K., (2012). Use of detoxified jatropha kernel meal and protein isolate in diets of farm animals. In: Makkar, H.P.S. (Ed.), Biofuel Co-Products as Livestock Feed–Opportunities and Challenges. FAO, Rome, Italy, pp. 351–378

Markel, E., English, B. C., Hellwinckel, C., & Menard, R. J. (2018). *Potential for pennycress to support a renewable jet fuel industry* (No. EEO-18-1-121). Hendun.

Malina, Robert, Megersa Abate, Charles Schlumberger, and Freddy Navarro Pineda. 2022. *The Role of Sustainable Aviation Fuels in Decarbonizing Air Transport*. World Bank.

Matsuda, F., Ishii, J., Kondo, T., Ida, K., Tezuka, H. and Kondo, A. 2013. Increased isobutanol production in Saccharomyces cerevisiae by eliminating competing pathways and resolving cofactor imbalance. Microbial Cell Factories, 12:119. <u>http://www.microbialcellfactories.com/content/12/1/119</u>

Milunović, Igor, Vera Popović, Nikola Rakaščan, Jela Ikanović, Vojislav Trkulja, Vuk Radojević, and Gordana Dražić. 2022. "Genotype× Year Interaction on Rye Productivity Parameters Cultivated on Sandy Chernozem Soil." *Genetika-Belgrade* 54 (2): 887–905. https://doi.org/10.2298/GENSR2202887M

Mofijur, M., Shams Forruque Ahmed, Zahidul Islam Rony, Kuan Shiong Khoo, Ashfaque Ahmed Chowdhury, MA Kalam, Irfan Anjum Badruddin, and TM Yunus Khan. 2023. "Screening of Non-Edible (Second-Generation) Feedstocks for the Production of Sustainable Aviation Fuel." *Fuel* 331:125879.

Molefe, Masego, Diakanua Nkazi, and Hembe Elie Mukaya. 2019. "Method Selection for Biojet and Biogasoline Fuel Production from Castor Oil: A Review." *Energy & Fuels* 33 (7): 5918–32. DOI: 10.1021/acs.energyfuels.9b00384

Montoya Sánchez, Natalia, Felix Link, Garima Chauhan, Cibele Halmenschlager, Hanan E. M. El-Sayed, Ranjit Sehdev, Rick Lehoux, and Arno de Klerk. 2022. "Conversion of Waste to Sustainable Aviation Fuel via Fischer–Tropsch Synthesis: Front-End Design Decisions." *Energy Science & Engineering* 10 (5): 1763–89. <u>https://doi.org/10.1002/ese3.1072</u>



Moser, B.R., Knothe, G., Vaughn, S. and Isbell, T. 2009. "Production and Evaluation of Biodiesel from Field Pennycress (Thlaspi Arvense L.) Oil." *Energy & Fuels* 23 (8): 4149–55.

Nafziger, E., Villamil, M., Niekamp, J., Iutzi, F. and Davis V. 2016. "Bioenergy Yields of Several Cropping Systems in the U.S. Corn Belt." *Agronomy Journal* 108(2), 559-565. <u>https://doi.org/10.2134/agronj2015.0203</u>

Navarrete, C., Estrada, M. and Martínez J. 2022. "Debaryomyces Hansenii: An Old Acquaintance for a Fresh Start in the Era of the Green Biotechnology." *World Journal of Microbiology and Biotechnology* 38 (6): 99. https://doi.org/10.1007/s11274-022-03280-x.

Ng, K. S., Farooq, D. and Yang A. 2021. "Global Biorenewable Development Strategies for Sustainable Aviation Fuel Production." *Renewable and Sustainable Energy Reviews* 150:111502. https://doi.org/10.1016/j.rser.2021.111502.

Ni, Z., Padilla, R., dos Santos Mello, L. and Nielsen, M. 2023. "Tuning Ethanol Upgrading toward Primary or Secondary Alcohols by Homogeneous Catalysis." *ACS Catalysis* 13 (8): 5449–55. https://doi.org/10.1021/acscatal.2c06322.

Ogunniyi, D. S. 2006. "Castor Oil: A Vital Industrial Raw Material." *Bioresource Technology* 97 (9): 1086–91.

O'Malley, J., Pavlenko, N. and Kim, Y. H. 2023. "Meeting the SAF Grand Challenge: Current and Future Measures to Increase US Sustainable Aviation Fuel Production Capacity." In . ICCT.

Panoutsou, C., Giarola, S., Ibrahim, D., Verzandvoort, S., Elbersen, B., Sandford, C., Malins, C., Politi, M., Vourliotakis, G. and Zita, V. E. 2022. "Opportunities for Low Indirect Land Use Biomass for Biofuels in Europe." *Applied Sciences* 12 (9): 4623.

Parisis, V., Tsave, O., Papanikolaou, Ch., Pantazopoulou, E. and Chatzidoukas, Ch. 2023. "Comprehensive Exploration of the Growth and Lipid Synthesis Phases of T. Oleaginosus Cultures Implementing Design of Experiments and Response Surface Methodology." *Bioengineering* 10 (12). https://doi.org/10.3390/bioengineering10121359.

Park, S-H, and Hahn, J-S. 2019. "Development of an Efficient Cytosolic Isobutanol Production Pathway in Saccharomyces Cerevisiae by Optimizing Copy Numbers and Expression of the Pathway Genes Based on the Toxic Effect of α -Acetolactate." *Scientific Reports* 9 (1): 3996.

Pasa, V. M.D., Almeida Scaldadaferri, C, and dos Santos Oliveira, H. 2022. "Main Feedstock for Sustainable Alternative Fuels for Aviation." In *Sustainable Alternatives for Aviation Fuels*, 69–102. Elsevier.

Persaud, L., Bheemanahalli, R., Seepaul, R., Reddy, K.R. and Macoon, B. 2022. "Low-and High-Temperature Phenotypic Diversity of Brassica Carinata Genotypes for Early-Season Growth and Development." *Frontiers in Plant Science* 13:900011.

Phippen W, Phippen M. (2010) Evaluation of field pennycress (Thlaspi arvense L.) populations [abstract]. Association for the Advancement of IndustrialCropsConference; September 19e22; Fort Collins, CO. New Crops: Exploring Diversity, Preserving our Future; http://www.aaic.org/10program.htm

"Phyllis Database." <u>https://phyllis.nl/Browse/Standard/ECN-Phyllis</u>

Priyadharshini, S, Archana, M., Angeline Kiruba, D., Priyadharshini, MN. and Malarvizhi. T. 2015. "Production of Isobutanol from Lactococcus Lactis Using Valine Catabolic Degradation Pathway." *Int. J. Eng. Technol. Sci. Res* 2:72–81.

Poltronieri, P. (2016). Alternative energies and fossil fuels in the bioeconomy era: what is needed in the next five years for real change. Challenges, 7(1), 11.

Putnam, D. H., Budin, J. T., Field, L. A., Breene, W. M., (1993). Camelina: a promising low-input oilseed. In: Janick, J., Simon, J. E. (eds), New Crops. Wiley, New York. pp. 314-322.



Qi, H., Li, S., Zhao, S., Huang, D., Xia, M. and Wen, J. 2014. "Model-Driven Redox Pathway Manipulation for Improved Isobutanol Production in Bacillus Subtilis Complemented with Experimental Validation and Metabolic Profiling Analysis." *PLoS One* 9 (4): e93815.

Rerop, Z.S., Stellner, N.I., Graban, P., Haack, M., Mehlmer, N., Masri, M., Brück, T.B. Bioconversion of a Lignocellulosic Hydrolysate to Single Cell Oil for Biofuel Production in a Cost-Efficient Fermentation Process. Fermentation 2023, 9, 189. https://doi.org/10.3390/fermentation9020189.

Robles-Iglesias, R, Naveira-Pazos, C., Fernández-Blanco, C., Veiga, M. and Kennes, Ch. 2023. "Factors Affecting the Optimisation and Scale-up of Lipid Accumulation in Oleaginous Yeasts for Sustainable Biofuels Production." *Renewable and Sustainable Energy Reviews* 171:113043.

Roozeboom, K.L, Wang, D., McGowan, A.R., Propheter, J. L., Staggenborg, S.A. and Rice, C. W. 2019. "Long-term Biomass and Potential Ethanol Yields of Annual and Perennial Biofuel Crops." *Agronomy Journal* 111 (1): 74–83.

Rossi, L., Fusi, E., Baldi, G., Fogher, C., Cheli, F., Baldi, A. and Dell'Orto, V. 2013. "Tobacco Seeds By-Product as Protein Source for Piglets." Open J Vet Med 3:73–78. doi:10.4236/ojvm.2013.31012

Roy, P., and Dahman, Y. 2023. "Mutagenesis of Novel Clostridial Fusants for Enhanced Green Biobutanol Production from Agriculture Waste." *Fermentation* 9 (2). <u>https://doi.org/10.3390/fermentation9020092</u>.

Sedbrook, J. C., Phippen, W. B. and Marks, M. D. (2014). New approaches to facilitate rapid domestication of a wild plant to an oilseed crop: example pennycress (Thlaspi arvense L.). *Plant Science*, *227*, 122-132.

Seepaul, R., Kumar, S., Iboyi, J.E., Bashyal, M., Stansly, T.L., Bennett, R., Boote, K. J., Mulvaney, M. J., Small, I.M. and George, S. 2021. "Brassica Carinata: Biology and Agronomy as a Biofuel Crop." *Gcb Bioenergy* 13 (4): 582–99.

Seepaul, R., Kumar, S., Sidhu, S., Small, I. M., George, S., Douglas, M. and Wright, D. L. 2023. "Effect of Tillage and Nitrogen Fertility on Growth, Yield, and Seed Chemical Composition of Rainfed Brassica Carinata." *Agronomy Journal* 115 (3): 1384–98.

Seepaul, R., Kumar, S., Iboyi, J. E., Bashyal, M., Stansly, T. L., Bennett, R., Boote, K. J., Mulvaney, M. J., Small, I. M., George, S., Wright, D. L., (2020) Brassica carinata: Biology and agronomy as a biofuel crop. GCB Bioenergy, 2021;13:582–599. DOI: 10.1111/gcbb.12804

Seepaul, R., Mulvaney, M.J., Small, I. M., George, S., Douglas, M. and Wright, D. L. 2020. "Carinata Growth, Yield, and Chemical Composition Responses to Nitrogen Fertilizer Management." *Agronomy Journal* 112 (6): 5249–63.

Shahabuddin, M., Alam, M. T., Krishna, B.B., Bhaskar, T. and Perkins, G. 2020. "A Review on the Production of Renewable Aviation Fuels from the Gasification of Biomass and Residual Wastes." *Bioresource Technology* 312:123596. https://doi.org/10.1016/j.biortech.2020.123596.

Sharma, R., Wungrampha, S., Singh, V., Pareek, A. and Sharma, M. K. 2016. "Halophytes as Bioenergy Crops." *Frontiers in Plant Science* 7:1372. doi: 10.3389/fpls.2016.01372

Shehab, M., Moshammer, K., Franke, M. and Zondervan, E. 2023. "Analysis of the Potential of Meeting the EU's Sustainable Aviation Fuel Targets in 2030 and 2050." *Sustainability* 15 (12): 9266.

Sriphuttha, C., Boontawan, P., Boonyanan, P., Ketudat-Cairns, M., Boontawan, A. Simultaneous Lipid and Carotenoid Production via Rhodotorula paludigena CM33 Using Crude Glycerol as the Main Substrate: Pilot-Scale Experiments. Int. J. Mol. Sci. 2023, 24, 17192. https://doi.org/10.3390/ijms242417192

Sindelar, A.J, Schmer, M. R., Gesch, R. W., Forcella, F., Eberle, C. A., Thom, M. D. and Archer, D. W. 2017. "Winter Oilseed Production for Biofuel in the US Corn Belt: Opportunities and Limitations." *Gcb Bioenergy* 9 (3): 508–24.



Singh, B., Singh, K., Shukla, G., Goel, VL., Pathre, U. V., Rahi, TS. and Tuli, R. 2013. "The Field Performance of Some Accessions of Jatropha Curcas L.(Biodiesel Plant) on Degraded Sodic Land in North India." *International Journal of Green Energy* 10 (10): 1026–40.

Singh, K., Singh, B., Verma, S.K. and Patra, DD. 2014. "Jatropha Curcas: A Ten Year Story from Hope to Despair." *Renewable and Sustainable Energy Reviews* 35:356–60. <u>https://doi.org/10.1016/j.rser.2014.04.033</u>

Siripong, W., Wolf, P., Puspowangi Kusumoputri, T., Downes, J. J., Kocharin, K., Tanapongpipat, S. and Runguphan, W. 2018. "Metabolic Engineering of Pichia Pastoris for Production of Isobutanol and Isobutyl Acetate." *Biotechnology for Biofuels* 11:1–16.

Slininger, P. J., Dien, B. S., Kurtzman, C.P., Moser, B. R., Bakota, E. L., Thompson, S. R., O'Bryan, P.J., et al. 2016. "Comparative Lipid Production by Oleaginous Yeasts in Hydrolyzates of Lignocellulosic Biomass and Process Strategy for High Titers." *Biotechnology and Bioengineering* 113 (8): 1676–90. https://doi.org/10.1002/bit.25928.

Soccol, C. R., Dalmas Neto, C. J., Thomaz Soccol, V., Bittencourt Sydney, E., Scopel Ferreira da Costa, E., Bianchi Pedroni Medeiros, A. and Porto de Souza Vandenberghe, L. 2017. "Pilot Scale Biodiesel Production from Microbial Oil of Rhodosporidium Toruloides DEBB 5533 Using Sugarcane Juice: Performance in Diesel Engine and Preliminary Economic Study." *Bioresource Technology* 223:259–68. https://doi.org/10.1016/j.biortech.2016.10.055.

Soorni, J., Kamal Kazemitabar, S., Kahrizi, D., Dehestani, A. and Bagheri, N. 2017. "Screening of Camelina (Camelina Sativa L.) Doubled Haploid Lines for Freezing Tolerance in the Seedling Stage." *Genetika* 49 (1): 173–81. <u>https://doi.org/10.2298/GENSR17011735</u>

Stefanoni, W, Latterini, F. and Pari, L. 2023. "Perennial Grass Species for Bioenergy Production: The State of the Art in Mechanical Harvesting." *Energies* 16 (5): 2303.

Van Dyk S. and Saddler J. IEA Bioenergy. 2021. "Progress in Commercialization of Biojet/Sustainable Aviation Fuels (SAF)." <u>https://www.ieabioenergy.com/wp-content/uploads/2021/06/IEA-Bioenergy-Task-39-Progress-in-the-commercialisation-of-biojet-fuels-May-2021-1.pdf</u>

Umakanth, AV., Datta, A., Reddy, B. S. and Bardhan, S. 2022. "Biomass Feedstocks for Advanced Biofuels: Sustainability and Supply Chain Management." *Advanced Biofuel Technologies*, 39–72. Elsevier. <u>https://doi.org/10.1016/B978-0-323-88427-3.00023-4</u>

Vollmann, J., Damboeck, A., Eckl, A., Schrems, H., Ruckenbauer, P., (1996). Improvement of Camelina sativa, an underexploited oilseed. In: Janick, J. (Ed.), Progress in New Crops. ASHS Press, Alexandria, VA, pp. 357–362.

Vollmann, J., Moritz, T., Kargl, Ch., Baumgartner, S. and Wagentristl, H. 2007. "Agronomic Evaluation of Camelina Genotypes Selected for Seed Quality Characteristics." *Industrial Crops and Products* 26 (3): 270–77.

Wang, J., Huang, C., Guo, K., Ma, L., Meng, X., Wang, N. and Huo, Y-X. 2020a. "Converting Escherichia Coli MG1655 into a Chemical Overproducer through Inactivating Defense System against Exogenous DNA." *Synthetic and Systems Biotechnology* 5 (4): 333–42.

Wang, N., Torralbo, F., Naumann, H. D. and Fritschi, F. B. 2023. "Tissue Composition and Higher Heating Values Differ among Six Bioenergy Grasses." *Industrial Crops and Products* 205:117437. <u>https://doi.org/10.1016/j.indcrop.2023.117437</u>

Wang, B., Tinga, ZJ, Zhao, M. Sustainable aviation fuels: Key opportunities and challenges in lowering carbon emissions for aviation industry. Carbon Capture Science & Technology 13 (2024) 100263. https://doi.org/10.1016/j.ccst.2024.100263



Warshay, B., Brown, J. J., and Sgouridis. S. 2017. "Life Cycle Assessment of Integrated Seawater Agriculture in the Arabian (Persian) Gulf as a Potential Food and Aviation Biofuel Resource." *The International Journal of Life Cycle Assessment* 22:1017–32. <u>https://doi.org/10.1007/s11367-016-1215</u>

Waypoint 2050. 2021. Available at: https://aviationbenefits.org/media/167417/w2050 v2021 27sept full.pdf

Weitz, S., Hermann, M., Linder, S., Bengelsdorf, F.R., Takors, R. and Dürre, P. 2021. "Isobutanol Production by Autotrophic Acetogenic Bacteria." *Frontiers in Bioengineering and Biotechnology* 9:657253.

Wess, J., Brinek, M. and Boles, E. 2019. "Improving Isobutanol Production with the Yeast Saccharomyces Cerevisiae by Successively Blocking Competing Metabolic Pathways as Well as Ethanol and Glycerol Formation." *Biotechnology for Biofuels* 12:1–15.

Xu, P., Qiao, K. and Stephanopoulos, G. 2017a. "Engineering Oxidative Stress Defense Pathways to Build a Robust Lipid Production Platform in Yarrowia Lipolytica." *Biotechnology and Bioengineering* 114 (7): 1521–30. https://doi.org/10.1002/bit.26285

Yu, H., Wang, N., Huo, W., Zhang, Y., Zhang, W., Yang, Y., Chen, Z. and Huo, Y-X. 2019a. "Establishment of BmoR-Based Biosensor to Screen Isobutanol Overproducer." *Microbial Cell Factories* 18:1–11.

Zanetti, F., Alberghini, B., Marjanović Jeromela, A., Grahovac, N., Rajković, D., Kiprovski, B. and Monti, A. 2021. "Camelina, an Ancient Oilseed Crop Actively Contributing to the Rural Renaissance in Europe. A Review." *Agronomy for Sustainable Development* 41:1–18.

Zanetti, F., Isbell, T.A., Gesch, R. W., Evangelista, R. L., Alexopoulou, E., Moser, B. and Monti, A. 2019. "Turning a Burden into an Opportunity: Pennycress (Thlaspi Arvense L.) a New Oilseed Crop for Biofuel Production." *Biomass and Bioenergy* 130:105354. <u>https://doi.org/10.1016/j.biombioe.2019.105354</u>

Zegada-Lizarazu, W., Parenti, A., Borghesi, A., and Monti, A. 2021. "Relay Cropping for Enhanced Food and Dedicated Lignocelulosic Feedstock Availability." In , 99–101. ETA-Florence Renewable Energies.

Zegada-Lizarazu, W, Parenti, A. and Monti, A. 2023. "BIOMASS POTENTIAL OF SUNN HEMP GROWN AS DOUBLE CROP FOLLOWING CONVENTIONAL WINTER CEREAL (S) IN A REDUCED SOIL TILLAGE SYSTEM." In , 47–49. ETA-Florence Renewable Energies.

Zegada-Lizarazu, W., João LN Carvalho, Parenti, A., Sarah Tenelli, Carlos Martín Sastre, Pilar Ciria, Myrsini Christou, Alexopoulou Efthymia, Antonio Bonomi, and Andrea Monti. 2022. "The Effects of Integrated Food and Bioenergy Cropping Systems on Crop Yields, Soil Health, and Biomass Quality: The EU and Brazilian Experience." *GCB Bioenergy* 14 (5): 522–38.

Zegada-Lizarazu, W., and Monti, A. 2012. "Are We Ready to Cultivate Sweet Sorghum as a Bioenergy Feedstock? A Review on Field Management Practices." *Biomass and Bioenergy* 40:1–12.

Zegada-Lizarazu, W, Parenti, A. and Monti, A. 2021. "Intercropping Grasses and Legumes Can Contribute to the Development of Advanced Biofuels." *Biomass and Bioenergy* 149:106086.

Zhang, A., Su, Y., Li, J. and Zhang, W. 2019. "Improving Isobutanol Productivity through Adaptive Laboratory Evolution in Saccharomyces Cerevisiae."

Zhang, Y, Lane, S., Chen, J-M., Hammer, S.K., Luttinger, J., Yang, L., Jin, Y-S. and Avalos J. L. 2019. "Xylose Utilization Stimulates Mitochondrial Production of Isobutanol and 2-Methyl-1-Butanol in Saccharomyces Cerevisiae." *Biotechnology for Biofuels* 12:1–15.


