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Bio-jet fuels from photosynthetic microorganisms: a focus on downstream processes

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Abstract

The aviation industry is a "hard to electrify" sector with limited possibilities for alternative fuels such as green hydrogen. Therefore, there is a call for sustainable fuel alternatives which is driving intensive research into bio-jet fuels (BJF) that could be used in aviation, without revamping the aircraft fleets and the aviation infrastructures. This review evaluates the potential of photosynthetic microorganisms, focusing on the most challenging downstream processes, i.e. bio-oil production and its catalytic upgrade. The main bottlenecks of such processes are identified, and the most recent solutions, offered to overcome them, are critically reviewed. Finally, a statistical analysis of the key characteristics of current BJF derived from microalgae and cyanobacteria is presented, along with a discussion on their suitability for the aviation industry and the primary areas for improvement.

Keywords: bio-oil; pyrolysis; hydrothermal treatments; lipid extraction; transesterification; catalytic upgrade.

1. Introduction

Jet fuels (JFs) are produced, almost exclusively, from fossil sources. Such an event makes the aviation industry extremely susceptible to oil price fluctuations [1]. In addition, it results one of the main responsible for the emission of greenhouse gases (GHGs) [2]. To overcome these issues, the aviation sector is paying increasing attention to renewable alternatives to replace traditional JFs. A promising solution might come from the adoption of so-called bio-jet fuels (BJFs) produced in advanced biorefineries, i.e., third and fourth generation biorefinery, relying on microalgae and genetically modified microalgae cultivation, respectively. Indeed, microalgal lipids represents an interesting source for biofuel production [3,4]. Microalgae boast an elevated photosynthetic efficiency which leads to a fast growth and, generally, to high biofuel production rates [5]. Moreover, they can be cultivated in saline or low quality waters, preserving the use of freshwater resources [6,7].

However, producing BJF from microalgae still faces economic hurdles [8], as the process must convert microalgal lipids, mainly comprising triacylglycerols (TAGs), into hydrocarbons, which are the components of conventional JFs [9]. Such a conversion is energy-intensive and costly, resulting in a steep price for BJF [10]. According to the projections of the Committee on Aviation Environmental Protection of the International Civil Aviation Organization (ICAO/CAEP), BJF prices may soar from $\approx \$1,220$ per ton in 2020 to $\approx \$1,460$ per ton by 2050, significantly higher than fossil JF prices. Therefore, microalgae based BJFs remain economically uncompetitive, necessitating further research and investment.

To correctly address future studies, it is fundamental to have a clear vision of the state of art of the different processes which contribute to the production of microalgae-based BJFs. While upstream processes, i.e. those related to microalgae cultivation, have been recently widely reviewed [9], a comprehensive review including all downstream processes, which greatly affect the final cost of the obtained BJF, is still missing. In more details, although several review papers concerning specific steps of the microalgae valorization into fuel have been published in the last 10-15 years [11–15], none of them include a systematic review of the entire downstream cycle, aimed at obtaining a fuel having the characteristics required by the aviation industry. The present paper intends to fill this gap, proposing a synthesis of the downstream processes which allow obtaining a bio-oil from an algal biomass, and providing a more detailed review of the final processes which allow to transform the microalgal bio-oil into a BJF.

2. Use of photosynthetic microorganisms to produce Bio-Jet Fuels

The selection of microalgal species for BJF production is crucial due to variations in growth rates, biomass yields, and lipid types. Factors like lipid content, cultivation conditions, and nutrients supply influence microalgal productivity. Previous reviews have examined BJF production process coupled with WW remediation [16]. Although many studies focus on biofuel production from microalgae, only few ones

specifically address its potential for aviation use [17]. Certain strains like *Isochrysis galbana* [18] and *Nannochloropsis* sp. [19] are prolific lipid producers. Fatty acids methyl esters (FAMES) composition of several microalgal strains used for biofuel production have been reported in a recently published dataset [20].

Promising strains for BJF formulation include *Nannochloropsis* sp, *Schizochytrium* sp, *Botryococcus braunii*, *Neochloris oleoabundans*, and *Nitzschia* sp., which offer significant advantages over other oils [21]. Strategies like nutrient starvation can significantly enhance lipid content, with *Nannochloropsis* sp. showing an 80% increase after 3 days of starvation [21]. *Schizochytrium* sp. is effective under heterotrophic conditions, producing high-value polyunsaturated fatty acids (PUFAs) and quality biofuel [22]. *Neochloris oleoabundans* has shown potential for BJF application using a two-step transesterification method [23]. Despite limited research on microalgae-based aviation fuel, strains like *Chlorella* sp. NT8a and *Pavlova salina* exhibit high growth rates, though *P. salina* has lower FAME content [17,24]. Marine strains like *Dunaliella salina*, *Tetraselmis suecica* and *Nannochloropsis* sp. BR2 show promising FAMES percentages despite lower growth rates [17]. Strains like *D. salina* and *Chlorella* sp. demonstrate significant lipid production potential, even with moderate growth rates [25].

Choosing the right microalgal species for BJF production is crucial due to variations in growth rates and lipid production. Enhancing lipid productivity while maintaining high biomass productivity is essential for the economic sustainability of BJF production [26]. Identifying parameters affecting lipid biosynthesis in microalgae and developing strategies to enhance this process is crucial. Stress-inducing conditions like extreme pH, temperature, radiation, osmotic stress, and heavy metals exposure can trigger lipid biosynthesis but often reduce biomass growth rates [27,28]. Balancing lipid content and biomass productivity is the key [29], but while stress management is a common approach in laboratories, its industrial adoption is rare. Identifying operational parameters and environmental factors influencing lipid accumulation in microalgae cells is essential for large-scale production [30].

3. Chemical and physical characteristics of fuels for the aviation sector

To correctly address the production of microalgal-based BJF, it is fundamental to know the physical and chemical characteristics for fuels to be used in the aviation sector.

The main physical characteristics of JFs are established by the American Society for Testing and Materials Standard Specification for Aviation Turbine Fuels ASTM D1655, which sets stringent safety standards to ensure optimal performances and operability. More specifically, this standard requires, among other: i) specific energy higher than 42.8 MJ kg⁻¹; ii) maximum temperature for 10% recovery equal to 205 °C; iii)

final boiling temperature equal to 300 °C; iv) density values ranging between 775 and 840 kg m⁻³; v) cetane number in the range 42-45; vi) freezing point for intercontinental flights between -40 and -47 °C; and vii) kinematic viscosity equal to 8 mm² s⁻¹.

The required physical characteristics, of course, are dependent on the chemical composition, which, therefore, must be optimized balancing different compounds [31]. The optimal distribution of hydrocarbons in JFs typically consists of 75–85 vol% of alkanes and 15–25 vol% of aromatics [32]. Oxygenated molecules, unsaturated hydrocarbons, and metals must be avoided due to their adverse effects on thermal stability, final boiling temperature and specific energy [33]. JFs should have minimal heteroatoms, as they decrease the specific energy value and can cause corrosion, instability, and NO_x and SO_x emissions. The presence of oxygen-containing groups can lead to hydrolysis, oxidation and polymerization reactions, affecting fuel quality and engine life [34]. High H/C ratios, characteristic of *n*- and iso-alkanes, offer the highest specific energy values [35], and are therefore preferred for JFs. Nonetheless the presence of cyclo-alkanes and aromatics, having lower H/C ratios, is also required to adjust the value of density and final boiling temperature of the produced JF. Moreover, aromatics are necessary for material compatibility, ensuring the proper swelling of nitrile O-rings and seals [21]. The hydrocarbon bulk composition of JFs also affects their ignition properties. The required CN, in fact, can be obtained only including *n*-alkanes in the fuel formulation. Long chained *n*-alkanes must be avoided to prevent freezing and high viscosity issues, while branched chain hydrocarbons and cyclo-alkanes mitigate these problems. The chemical composition also affects the combustion emissions. Linear alkanes burn cleanest, followed by cyclo-alkanes, while aromatics produce more soot and smoke.

4. Biodiesel production by lipid extraction

As indicated in Figure 1, one of the first downstream processes applied for BJF production from microalgae is lipids extraction, eventually preceded by cell disruption.

Figure 1

In conventional extraction processes, the efficiency of lipids extraction processes, carried out using appropriate solvents, depends on solvent choice, cell-solvent contact time and biomass water content. Innovative solvents, such as ionic liquids, offer the advantages of low reagent volatility, high capacity to dissolve organic molecules, reduced reaction times, and possibility of recovering and reusing the chemicals. However, their high cost and toxicity limit a potential large-scale use [36]. On the other hand, supercritical fluids, which exhibit both liquid and gas characteristics, have a much reduced or no toxicity, and adjustable solvation capabilities. Nonetheless high power requirements and elevated costs associated to a large-scale use, limit their application [37].

4.1 Cell disruption pre-treatments

The efficiency of conventional extraction processes can be increased applying preliminary pre-treatments to disrupt microalgae cells [38] [39].

Pre-treatments, indeed, enhance lipid extraction rates and preserve the chemical properties of extracts, addressing shortcomings such as large organic reagent consumption, environmental pollution, and prolonged extraction times [39].

Among physical pre-treatments, those based on high (above 100 °C), mild (50-100 °C) and freezing temperatures (below 0 °C) have been extensively tested [12,40–42]. Freezing temperatures (below 0 °C) can be used either applying a series of freezing/thawing cycles leading to cell disruption by formation of ice crystals and cell expansion upon thawing [43] or applying a slowly decreasing temperature (down to -40 °C), associated with a high pressure (around 1 kPa) [13,44]. Simultaneous application of temperature and pressure are characteristic also of the processes using high temperatures, such as the hydrothermal cell disruption, which uses temperatures above 100 °C and pressures over 220 bars [45]. Steam explosion, in turns, exposes algal biomass to saturated vapor pressure at high temperature (140-180 °C) for up to one hour, before a rapid depressurization which breaks the cells and releases the intracellular content [46]. Finally, microwaves pre-treatments, conducted at a frequency of 2450 MHz, heat cells internally, causing their disruption. Generally, these pre-treatments results both effective and robust, and are easy to scale-up, although the effectiveness is also dependent on the microalgae strain [47]

Cold mechanical pre-treatment methods using bead mills, presses, and low frequency waves (ultrasonication) are also widely used. Bead milling makes use of agitated small beads, made of glass, ceramic, plastic, or steel, to collide with cells and disrupt them [46]. Pressing, in turns, makes use of a compressive force, applied by expellers, pistons and screws [48,49]. In case of the so-called French press the microalgae are forced through a valve under high pressure, which causes cell disruption. Finally, ultrasonication uses low frequency waves (18–40 kHz) to produce cavitation breaking microalgal cells even at low concentrations [47].

Another promising physical pre-treatment utilized for cell disruption is based on the application of a pulsed electric field [50] which induces electroporation, increasing cell wall permeability and potentially leading to cell wall damage. This method can be tuned for reversible or irreversible cell wall disruption, depending on the extent of the electroporation applied.

Chemical disruption, instead, is obtained using a large variety of compounds, which selectively interact with cell wall constituents [12,51]. These methods offer more selectivity than physical ones [50] and are mainly influenced by: i) type and concentration of chemicals; ii) process temperature; iii) reaction time; iv) biomass concentration; and v) microalgae species. Advantages of chemical pre-treatments include low

energy consumption, high cell disruption efficiency, and easy upscaling. Drawbacks include high cost of used chemicals and potential impacts on the quality of the end-products [52].

Acid and alkaline chemical pre-treatments generally use H_2SO_4 and NaOH and, therefore [53], can be disadvantageous for industrial applications due to inhibitors generation, equipment corrosion, challenging chemical recovery, and high operational costs. In addition, acids and alkaline solutions have low selectivity, releasing multiple components and complicating subsequent separation. Enzymatic hydrolysis, in turns, make use of an appropriate enzyme (lipase, protease, cellulase, glycosidase, amylase, xylanase, peptidase) to break down cell wall components into monomeric sugars and amino acids [54]. Compared to acid/alkali pre-treatment, enzymatic hydrolysis is more advantageous in terms of selectivity, conversion yield, operating conditions, energy requirements, and scalability. However, it produces inhibitors, requires long incubation times, and involves high enzyme recovery costs. Oxidizing agents such as H_2O_2 or O_3 can also disrupt cell wall constituents [39]. In Fenton's reaction, ferrous and/or ferric cations catalytically decompose H_2O_2 to generate powerful oxidizing agents that attack the cell wall, though there is a risk of oxidizing target compounds if reaction times are not short [55]. More recently ozonolysis has gained popularity [56] due to its advantages, including low inhibitor production, no chemical or liquid phase requirements, mild conditions, on-site O_3 production, and easily degradable by-products. However, high operational and equipment costs, and the toxic, flammable, corrosive, and highly reactive nature of O_3 are significant drawbacks [57]. The efficiency of the main lipid-extraction methods, associated to various pre-treatments is summarized in in Figure 2.

Figure 2

The Figure illustrates significant variability in the data associated with certain extraction techniques. This variability can be attributed to several factors. Firstly, studies employing the same extraction technique often utilize different microalgae strains, each with distinct cell size and cell wall compositions that influence solvent diffusion, as well as varying lipid content and composition. Moreover, variations in operational parameters of each extraction technique, including pressure, temperature, solid-to-liquid ratio, extractant concentration, residence time and others, further contribute to these discrepancies. Overall, these factors account for the observed variations in extraction efficiency and yield.

Conducting a comparative analysis of the dataset in Figure 2 was particularly challenging due to the substantial variation in sample sizes across different extraction techniques. While the Tukey test can be applied to unequal sample sizes, its robustness dramatically diminishes when the sample sizes differ significantly. To address this, we carefully selected data from the original dataset that would allow for a meaningful comparison. Specifically, extraction methods with extremely limited data availability, such as

pressurized Liquid Extraction (PLE), Supercritical (SC) -CO₂, and Organic Solvent (OS), were excluded from the analysis. Subsequently, as many non-null values as possible were retained using pairwise deletion, and outliers were removed for each extraction method under consideration.

The ANOVA test conducted on this refined dataset yielded a p-value of 0.062, indicating no strong statistical evidence of differences among the evaluated extraction techniques (AT, BB, MAE, PEF, UAE). Furthermore, a Tukey's post-hoc test performed on the same dataset produced p-values greater than 0.05 for all pairwise comparisons, confirming that none of the tested methods significantly differed from one another in terms of extraction efficiency.

4.2 Upgrading processes

Once extracted from the microalgal biomass, the lipids are upgraded into biodiesel through transesterification. The process consists, generally, in the catalytic reaction of acylglycerols (mainly TAGs) with an alcohol (for instance methanol) to produce a mixture of glycerol and fatty acids methyl esters (FAMES). FAMES are then separated from the mixture and recovered. Glycerol can also be valorized, offsetting the cost of the process, although the presence of other contaminants in the algae lipid feedstock may increase it substantially. An illustrative - but not exhaustive - example of the transesterification reaction is illustrated in Figure 3.

Figure 3

Catalysts used for the transesterification reaction can be classified as homogeneous (mainly H₂SO₄, HCl, and H₃PO₄, NaOH, KOH, KOCH₃), or heterogeneous (mainly CaO, SiO₂, TiO₂, CH₂O₂, cellulose, and biochar), based on their chemical nature [58,59]. Homogeneous acidic catalysts are widely used for biodiesel production from feedstock having a high free fatty acid (FFA) content. FFA content, in fact, is reduced through acid esterification followed by transesterification of acylglycerols in the presence of a basic catalyst. Transesterification can also be mediated by enzymes, assisted by microwaves or ultrasound, or conducted through reactive distillation and hydrothermal liquefaction [60–62].

In all cases, transesterification allows obtaining biodiesel yields higher than 85% [58,63], for example, obtained a biodiesel yield of 98.3% from *Scenedesmus obliquus* adopting a transesterification process, 6 h long, mediated by a 15% heterogeneous catalyst (Cr–Al) at 80 °C, using an alcohol/oil molar ratio of 20:1. Sivaramakrishnan and Incharoensakdi [60], in turns, obtained a yield of 88% from *Botryococcus* sp. through an enzymatic ultrasound assisted transesterifications, conducted at 500 °C applying a 200 W power ultrasound for 6 h, using, as catalyst, a 10 wt% *Candida antarctica* lipase B immobilized on Celite. A similar yield (87.7%) was obtained by Karimi [62] from *Chlorella* sp. using H₂SO₄ as homogenous catalyst.

The reaction took place at 24 °C in 100 min, applying a 150 W power ultrasound, and using an alcohol/oil molar ratio of 83:1. A 86.4% biodiesel yield was reported by Pandit and Fulekar [59] from *Acutodesmus obliquus*, using a heterogeneous catalyst (1.7 wt% CaO, synthesized from eggshell). The reaction was carried out in 216 min at 70 °C with an alcohol/oil (wt vol⁻¹) ratio of 10:1. Finally, Torres et al. [61] obtained a biodiesel yield around 85.5% from *Nannochloropsis gaditana* using two vacuum distillation units.

5. Bio-oils production by thermal treatments

Thermal treatments represent an alternative to lipid extraction for bio-oil production from microalgae. These treatments are generally conducted in absence of air and include both pyrolysis and hydrothermal processes (HTPs). Pyrolysis uses dry biomass, while HTPs use wet biomass (75-95% water). HTPs can be further distinguished into hydrothermal carbonization (HTC), operating a 150-250 °C under mild pressure (< 2 MPa), and hydrothermal liquefaction (HTL), operating at sub-or super-critical temperatures and pressures [15,64–66]. While HTC results in the production of a solid fuel indicated as biochar [67,68], HTL results in the production of a liquid fuel referred as bio-crude, along with a biochar, aqueous products, and gases as secondary by-products [69,70]. Therefore, only HTL can be used to produce bio-oils.

5.1 Hydrothermal processes

As said, HTL allows processing wet biomass directly, avoiding the energy-intensive drying step and producing no SO_x emissions [71]. Water, used as a solvent, is non-toxic, cheap, and environmentally safe, and acts as both catalyst and reactant [72]. However, resulting algal bio-crudes are rich in O (10-15%) and N (5-8%), and, therefore, are suitable for the production of polymers and lubricants but difficult to be used as fuels without further up-grading, because of their acidity, high water content, corrosivity characteristics, poor combustion performance, and low H/C ratio [73].

Several studies have been conducted on algal biomass using HTL, obtaining promising results [74,75]. The process has been applied both to freshwater strains, such as *Chlorella* sp., *Nannochloropsis* sp., and *Spirulina* sp. using, as catalyst, Na₂CO₃ and formic acid [76], and to marine strains, such as *Picochlorum celery* [75], obtaining a bio-crude yield of 0.33 g g⁻¹ algae and favourable physical properties for the production of BJFs. Mixed-culture algal biomass cultivated in wastewater treatment facilities have been tested too, obtaining a bio-crude rich in hydrocarbons and fatty acids (FAs) [74].

It is worth mentioning that a comprehensive report by the Argonne National Laboratory, and the Pacific Northwest National Laboratory in USA has highlighted the advantages of HTL over lipid extraction for algal biofuel production, qualifying it as a powerful biofuel and biomass-based biodiesel production approach under the Bioenergy Technology Office objectives, pursued in the frame of the Multi-Year Program Plan [77].

5.2 Pyrolysis

Contrary to HTP, pyrolysis is applied on dry biomass. Pyrolysis is an endothermic process conducted in the absence of oxygen at atmospheric pressure and medium to high temperature (300–700 °C), which results in the production of liquid, gas and solid compounds [78]. The liquid phase, obtained by condensing the vapor after having separated the solid residues, includes a bio-oil, rich in high molecular weight compounds, and an aqueous (acid) extract. The gas phase consists of low molecular weight compounds with moderate vapor pressure at room temperature, and the solid phase, known as biochar, is a porous matrix similar to charcoal [79]. The yields of bio-oil, gases and biochar depend on operating parameters such as temperature, heating rate, pressure, residence time, flow rate of inert gas, and the type of pyrolysis (slow, fast, or flash) [80].

Asiedu et al. [81] reported significant triglycerides and low carbohydrates and proteins levels from pyrolysis of *Chlorella vulgaris*. Thermo Gravimetric Analysis demonstrated that the oil-laden biofuel intermediate was decomposed at 400 °C and the pyrolysis of the intermediate mostly produced alkenes (gaseous hydrocarbon) with a high heating value (HHV) up to 48.5 kJ mol⁻¹. To characterize the oil extracted from the intermediate, it was subjected to catalytic transfer hydrogenation (CTH). The principal liquid products were alkenes (5%), aromatics (5%), *n*-alkanes (14%), cyclo-alkanes (35%) and iso-alkanes (41%).

As pyrolysis is a high endothermic process, the cost effectiveness of a system to produce BJFs via pyrolysis is not granted. Liu and Wang [82], for example, demonstrated that, in order to exploit a pyrolytic process for BJFs production in a plant having a capacity of 600 ton d⁻¹, the minimum BJF selling price cannot be lower than \$3.21 L⁻¹.

6. Upgrading processes for BJF production

The biodiesel obtained from the transesterification of the lipids extracted by microalgae as well as the bio-oils produced by microalgal biomass thermal treatment are not suitable for direct use as JF, having low specific energy (between 34-42 MJ kg⁻¹), high kinetic viscosity (3-23 mm² s⁻¹), high density (858-958 kg m⁻³), low C+H content (8-43 w%), and high total acidity (0.2-0.8 mg_{KOH} g⁻¹). All these characteristics are related to the presence of long chain esters of FAs (mainly C8-C16) presenting multiple unsaturation points and high oxygen content (approximately 11% by weight) [83]. Even though some studies evidenced the possibilities of selecting and improving microalgal strains, cultivation and harvesting conditions for adapting C atom distribution [17,84], the microalgal biodiesel would require anyway a further upgrade before being used as JF, mainly due to the high unsaturation degree and O-content. Indeed, because of the presence of a large amount of polyunsaturated fatty acids (PUFA) containing the bis-allylic hydrocarbon molecules, microalgal biodiesel is subject to autoxidation reactions. R-H groups, in fact, in contact with

oxygen, are converted into R–O–OH groups and then oxidised to carbonyls and carboxylic acid compounds. Consequently, microalgal biodiesel is highly unstable. In addition, the hygroscopic nature of biodiesel is responsible for water adsorption of the fuel and can promote the growth of acetogenic bacteria leading to corrosion of metal components in pipes and engines. For all these reasons, before being converted into BJF microalgal biodiesel must be first subjected to a hydrogenation process aimed at reducing the unsaturation degree of the FAMES. Such a treatment allows increasing the oxidative stability, the energy density, and the viscosity of the fuel. Nonetheless, the O content remains still high due to the O presence in the carbonyl groups. Accordingly, further processes of O removal, cracking, and isomerization reactions are needed to upgrade the biodiesel to BJF. Double bond hydrogenation and O removal reactions often are combined in one step for economic aspects. Then, a mixture containing saturated linear hydrocarbons is upgraded by cracking, and isomerization reactions to achieve necessary composition and properties of BJF.

Hydrotreatment (HT) is the catalytic conversion process that includes unsaturated bond hydrogenation, oxygen removal, hydrocracking, and hydroisomerization reactions of feedstock oil in the presence of H₂ at high pressure (50-100 bar) and temperature (300-450 °C).

The first reaction, occurring at low temperature (110-150 °C) is the saturation of -C=C- double bonds of TAG and FAME molecules [85]. Afterwards, saturated feedstock molecules undergo hydrogenolysis reaction in the presence of H₂. TAG hydrogenolysis is also called depropanation. In this step, ester bonds are cleaved with H₂ leading to the formation of FFAs. Gradual cleavage of ester bonds is observed with di- and mono-glycerides as intermediates. Three FFAs and propane are obtained as products. When hydrogenated FAME is used as feedstock, FFA and methane are produced by hydrogenolysis [86]. Carboxylic groups of FAs moieties stay untouched in hydrogenolysis reactions. Thermochemical data investigated the TAG and FFA chemical reactions under an H₂ atmosphere, revealing that carboxylic group reduction of FA moiety in TAG molecule is less favorable than hydrogenolysis reaction [87]. Therefore, O removal reactions proceed only when TAG and FAME molecules are transformed into FFA.

Many studies deal with HT upgrading of algal derived FAMES into hydrocarbon mixture. Viêgas et al., [88] investigated the HT processing of FAMES derived from *Chlorella* alga lipids with H₂ at 3 MPa and 300 °C, over 5% Pd supported on C (5% Pd/C) and 5% Ni supported on zeolite (5% Ni/HY-80). The results obtained after 150 min revealed that the Ni/HY-80 catalyst had a higher activity than the Pd/C, being able to convert ~95% of FAMES into hydrocarbons consisting mainly of hexadecane (C16), heptadecane (C17) and octadecane (C18). However, hydrocarbon mixture still contained FFAs and alcohols meaning that Ni catalyst did not provide full conversion of feedstock into renewable hydrocarbons.

[22] produced renewable hydrocarbons and value-added chemicals from *Schizochytrium* sp. algae extract using HT. Interestingly, the obtained lipid extract contained large amount of PUFAs, which causes rapid catalyst deactivation *via* heavy coke formation under HT process. Therefore, the authors executed

transesterification of algal extract and separated PUFA esters by short-path distillation prior to catalytic HT. Afterwards, remaining FAME were catalytically deoxygenated over 1% Pt/ γ -Al₂O₃ catalyst at 360 °C and 20 bar H₂ pressure in fixed-bed reactor. Oxygen was removed by decarbonylation and decarboxylation pathways producing CO and CO₂ gases in the presence of Pt catalyst. High liquid paraffin yield (75.9 %) was obtained in catalytic HT process without significant catalytic deactivation.

Some scientific reports focused on algal lipid direct catalytic HT into renewable hydrocarbons. Microalgae oil consisting mainly of neutral lipids was converted into alkanes with yield 76-78% under HT conditions in the presence of 10% Ni/H-Beta [89] and 10% Ni/ZrO₂ [90] catalysts in batch type autoclave. Noble metal supported 1% Pt/Al₂O₃, 0.5% Rh/Al₂O₃ and bimetallic presulfided NiMo/ γ -Al₂O₃ catalysts were tested on microalgae oil extracted from *Nannochloropsis salina* strain [91] utilizing continuous flow micro reactor. All catalysts were active on saturation and oxygen removal reactions of feedstock and alkane mixture in the range from C13-C20. Both noble metal catalysts favoured the hydrodecarboxylation/hydrodecarbonylation route, and their selectivity was not affected by the experimental conditions. However, the selectivity of bimetallic catalyst changed from hydrodeoxygenation pathway to hydrodecarboxylation/hydrodecarbonylation at reduced H₂/Oil ratio and residence time. Moreover, Pt and Rh catalysts exhibited higher resistance against deactivation and the coke formation on catalysts specific surface decreased in following order: Ni > Mo > Pt > Rh. The highest hydrocarbon yield of 76.5% was obtained over Pt catalyst.

Podar et al. [92] tested a different sulfided bimetallic CoMoP/Al₂O₃ catalyst on hydroprocessing of neutral lipids extracted from *Nannochloropsis* sp. algal biomass. In batch type autoclave full conversion was achieved at 375 °C and 50 bar pressure in the presence of CoMo catalyst and product consisting of 6% gasoline, 65% kerosene and 29% diesel was obtained.

Due to the exploitation at high altitude and low temperature, BJT must contain explicit chain length (C8-C16) linear hydrocarbons. Such hydrocarbons must be characterized by a high degree of branched analogs to meet standard-compliant cold flow properties (freezing point), kinematic viscosity, and density. Moreover, cold flow properties of linear alkanes are poor, but can be significantly improved by higher *iso*-alkane content, especially multi branched alkanes, which exhibit lowest freezing points [1]. Long chain *n*-hydrocarbons are transformed into shorter length linear and branched alkane mixture by catalytic hydrocracking and hydroisomerization reactions. These reactions require similar operating conditions as oxygen removal reactions, and specific bifunctional catalysts to obtain hydrocarbons with appropriate composition. Therefore, hydrocracking and hydroisomerization can be combined in one HT step with unsaturated bond hydrogenation and O removal reactions or can be performed in an additional upgrading steps [86]. Both solutions have some advantages and drawbacks. A single stage can decrease the operational cost and the number of required procedures. However, compared to two-stage process, higher metal loading

catalyst is necessary (up to 2% in noble metal case), product selectivity control is difficult, and catalyst stability is lower.

Pre-treatment of feedstock (pre-hydrogenation, pre-distillation) prior to HT process can significantly improve bifunctional catalyst life span and increase hydrocarbon yield. Pre-hydrogenation can be carried out under milder reaction conditions (150-250 °C) in the presence of inexpensive monometallic catalyst saving overall costs of the process [93].

Two-stage process is costly, but offers higher BJF yields, longer catalyst life and suppression of aromatic species formation [1,86].

Kim et al. [22] investigated 1% Pt supported on mesoporous zeolite (Pt/mesoBEA) catalyst for upgrading of hydrocarbon mixture obtained from FAEEs by catalytic oxygen removal hydrotreating reactions. Hydrocracking-hydroisomerization reaction was conducted in fixed-bed reactor at 220–240 °C temperature and 20 bar pressure obtaining BJF with 20.4 wt% yield. Robota et al. [94] used a 0.5% Pt/USY for upgrading of diesel range hydrocarbons obtained by HT of algal lipids. Although alkane mixture (C5-C18) with *iso*/*n* = 1 was obtained at 258 °C and 5.5 MPa, product solidified near ambient temperature. Therefore, a solvent dewaxing procedure, was applied and resulted in a product that remained liquid at –20 °C. Kruger et al. [95], instead, tested a 1% Pt/SAPO-11 catalyst. Hydroconversion was carried out at 350 °C and 3.45 MPa pressure and the obtained liquid yields were in range 77-81% with *iso*-paraffin content reaching 30-50%. Cloud points for obtained green fuels were -12 and -21 °C, which corresponded more to diesel than JF properties.

Cheng et al. [96] reported an example of bifunctional catalysts applied to one-pot HT including double bond hydrogenation, O removal, hydrocracking and hydroisomerization reactions of *Nannochloropsis* microalgal biodiesel. The authors loaded phosphotungstic acid H₃PW₁₂O₄₀ (HPW) on Ni-based hierarchical mesoporous zeolite Y. 10% Ni/meso-YHT reaction of algal biodiesel was performed in fixed bed continuous flow system at 255 °C and 2 MPa pressure and the overall BJF selectivity reached 63.1% in which *iso*-alkane selectivity augmented to 20.5%. Moreover, density and HHV of produced liquid product met ASTM standards for JF, but the kinematic viscosity and freezing point was beyond standard requirements.

Cheng et al. [97] investigated the possibility to use 10% Ni supported on sulfonated mesoporous Y zeolite (Ni/meso-Y) to promote hydrocracking of *Nannochloris* algae derived FAMES into hydrocarbons suitable for the aviation sector. The conversion was conducted on a fixed bed continuous flow reaction system at 275 °C and 2 MPa pressure and microalgae biodiesel conversion reached 99.7% and JF range alkane selectivity was 52.3%.

Verma et al. [98] investigated two-stage and one-stage HT processes for algal lipid extract conversion into BJF. Micro-mesoporous 0.75% Pt-SAPO-11 catalyst was tested in second-step as hydrocracking and

hydroisomerization catalyst. Hydrocarbon mixture obtained in first HT step was upgraded at 375 °C and under 50 bar pressure. Pt/SAPO-11 produced jet range hydrocarbons with 33% yield, high conversion (83%) and *iso/n* ratio = 4.2. However, low yield and consumption of resources for two HT processes execution, indicates that single step hydroconversion process could be more beneficial and economical. Authors synthesized new acidic zeolitic ZSM-5 support with hierarchical structure and intra-crystalline mesoporosity. Obtained catalyst 5% NiO-18% MoO₃/H-ZSM-5 converted algal lipids into BJT range hydrocarbons (C₉-C₁₅) with high yield (79%) and conversion (98%), and moderately high isomerization selectivity (*iso/n* = 2.5) under single step HT (410 °C, 50 bar) process. Moreover, obtained alkane mixture had suitable aviation fuel properties (freezing point -55°C, density 0.78 g ml⁻¹ at 15 °C etc.).

7. Characteristics of microalgal BJT obtained through the different techniques

Different types of parameters are reported in the literature to assess the goodness of the BJT obtained through different adopted processes. Most frequent considered parameters include: i) elemental composition; ii) specific energy; iii) density; iv) viscosity; v) number of acids; and vi) moisture. Less frequently are reported also: vii) freezing and flash points; viii) cetane number; ix) hydrocarbon distribution; x) metal content; and xi) the produced emissions [17].

Table 1 summarizes the aggregated data of some of these parameters (mean values and 95% confidence interval), categorised according to the process used for bio-oil production (extraction and transesterification, BD; pyrolysis, BP; hydrothermal treatment, BHT) and potential BJT production (catalytic up-grading of BD and BHT). No sufficient data have been found for catalytic upgrade of pyrolysis oil. Therefore, they have been excluded from the Table.

Table 1

Reported data were analysed to verify the presence of correlations ($r > 0.9$) between physical properties and chemical characteristics. The most significant correlations were found between the specific energy and C, O and water content (Figure 4).

Figure 4

In more details, specific energy was found to be directly correlated with C content and inversely correlated with O and water content. In addition, it was found a direct correlation between viscosity and total acidity, and between total acidity increases and water content. Finally, it was found an inverse correlation between water content and C content and a direct correlation between water content and O content (data not shown). These correlations confirmed that the O content should be minimised through catalytic up-grade procedures [20].

A further analysis of literature data, summarized in Figures 5a, 5b, 5c and 5d, allowed identifying the differences existing among potential BJFs (HBD, HBHT), and fuel precursors (BD, BP, BHT) obtained through different techniques.

Figure 5

Despite the considerable variability, attributable to the different strains analyzed, and the slight variations in operating conditions across studies using the same BJF production technique, valuable insights could be derived from the analysis.

Indeed, Figure 5a shows that the specific energy of bio-oils obtained from hydrothermal treatment (35-37 MJ kg⁻¹) presents values similar to those obtained by extraction and transesterification (34-42 MJ kg⁻¹), while pyrolytic oil is characterised by a lower energy content (23-28 MJ kg⁻¹). Process-aggregated data clearly show the effect of up-grade treatment in increasing the specific energy of bio-oils (42-47 for MJ kg⁻¹ for HDB, and 43-44 MJ kg⁻¹ for HBHT) with the production of BJF that, after the catalytic hydrotreatment, meets the minimum requirements for JF (Figure 5a).

Similarly, Figure 5b shows that the kinematic viscosity is extremely high and heterogeneous for bio-oils obtained from hydrothermal treatment (89-340 mm² s⁻¹) in comparison to those obtained from pyrolysis (13-89 mm² s⁻¹), and that biodiesel obtained by extraction and transesterification presents the lowest values (3-23 mm² s⁻¹). After catalytic up-grade, the kinetic viscosity of fuels from BD meets the minimum requirements (1-10 mm² s⁻¹), while fuels from BHT still presents too high values, which could be critical for their future approval. Data concerning O content in the different bio-oils (Figure 5c) evidence a high and wide distribution in pyrolytic oils (21-30%w) in comparison with products obtained by extraction and transesterification (14-24 %w), and by hydrothermal treatment (10-13 %w). Noticeably, even after the up-grade, O content is too high (average 5%w for HBD and 2.5%w for HBHT) considering the minimum requirement for C+H w% fixed at 99.5%. Densities of crude bio-oils (Figure 5d) present values higher than the optimal range (858-958 kg m⁻³ for BD, 1100-1219 kg m⁻³ for BP, and 909-1066 for BHT). Although these values are sensibly reduced after up-grading (736-931 kg m⁻³ for HBD, and 772-911 kg m⁻³ for HBHT) they remain still too high, requiring a further up-grade to be within the target range. The sulphur content is another parameter commonly evaluated for bio-oils. Total S can be extremely high in BD and is still quite high after up-grading, requiring further optimisation (Figure 7e). Other parameters, requested as minimum requirements for BJF, are generally not addressed or reported in literature data, thus needing further investigation by researchers to obtain a representative view of possible issues in future BJF certification procedures.

The statistical analysis performed for the dataset presented in Figure 2 was similarly applied to the dataset in Figure 5. In this analysis, production techniques with insufficient data points were excluded to ensure the validity of the statistical comparison. Consequently, BJF production techniques with limited data

availability were removed from consideration. To maximize the retention of non-null values, pairwise deletion was employed. Outliers were identified and excluded prior to conducting the statistical tests. The analysis involved an ANOVA test followed by Tukey's post-hoc comparisons.

Regarding the influence of production techniques on the specific energy of the resulting BJF (Figure 5a), a statistically significant difference was observed among all the compared techniques, namely HBHT, BHT, and BP. The results indicate that the HBHT technique produced BJFs with the highest specific energy, followed by BHT and BP. Due to insufficient data, the BD and HBD techniques were excluded from this analysis.

For the effect of production techniques on kinematic viscosity (Figure 5b), only BD and HBHT could be statistically compared via Tukey's test. The analysis revealed no significant difference in kinematic viscosity between BJFs produced using these two techniques.

The findings for BJF oxygen content (Figure 5c) were consistent with those observed for specific energy. As with the previous analysis, BD and HBD were excluded due to sample size limitations. The ANOVA test yielded an extremely low p-value, indicating strong statistical significance. Tukey's post-hoc test results demonstrated significant differences among all production techniques considered (HBHT, BHT, and BP). These findings further corroborate the results in Figure 4a, confirming that production techniques associated with higher oxygen content also yielded BJFs with lower specific energy, and vice versa.

For BJF density (Figure 5d), a statistical comparison was only feasible between the BD and BP techniques, as the remaining techniques had insufficient data for inclusion. The analysis identified a statistically significant difference ($p < 0.05$), with BP producing BJFs of higher density than BD.

Finally, in the case of sulfur content (Figure 5e), a comparative analysis was conducted between BP and BHT, as only these techniques had sufficient data for statistical evaluation. The results revealed significantly higher sulfur content in BJFs produced using BHT compared to BP.

Overall, this comprehensive statistical assessment highlights the impact of different production techniques on key physicochemical properties of BJFs, providing valuable insights into the relationships among specific energy, oxygen content, kinematic viscosity, density, and sulfur content.

8. Key challenges in bio-jet fuel production and commercialization

The development and large-scale adoption of BJFs face several technical, economic, and regulatory challenges that still hinder their widespread commercialization. These challenges concern the entire production chain, from feedstock supply to fuel certification, and require technological advancements, economic incentives, and policy support to overcome [10].

Among the primary technical barriers there are feedstock availability and processing efficiency. While photosynthetic microorganisms are a promising feedstock due to their high lipid content and non-

competition with food crops, their large-scale cultivation requires significant water, and nutrient inputs, potentially limiting their sustainability [99]. In this regard the use of wastewater as nutrient source could lower biomass production costs. Additionally, variations in biomass composition can impact fuel yield and quality, necessitating optimized cultivation and harvesting techniques. In this regard, the optimization of the engineering of microalgal cultivation, including photobioreactor design and operation, can positively influence biomass selection and lipid productivity [9].

Downstream processing of microalgal biofuels presents further challenges. As said, lipid extraction, which is a key step in bio-oil production, currently relies on expensive and energy-intensive techniques. These processes often require large amounts of solvents or operate under high temperatures and pressures, making them inefficient and costly [99]. To address these issues, research should focus on cell disruption techniques that facilitate intracellular lipid release and methods to reduce the energy demand, such as integrating renewable energy sources into the process or developing novel, wet techniques to avoid the costly dewatering stage of microalgal biomass. Moreover, the adoption of green solvents can play a key role.

Once extracted, bio-oils must be upgraded to meet the stringent requirements of aviation fuels. Catalytic hydrotreatment improves the energy content and reduces viscosity, yet high oxygen content remains a major challenge. Oxygen-rich fuels tend to have higher viscosity and water content, which negatively impact fuel performance and storage stability. Effective deoxygenation, hydrocracking, and hydroisomerization processes are the key for converting bio-oils into hydrocarbons within the desired C8-C16 range suitable for aviation [100]. However, even after upgrading, oxygen levels in bio-jet fuels often remain above acceptable thresholds, requiring further refinement. The selection of high-performance catalysts, such as Pt/Al₂O₃, Rh/Al₂O₃, and NiMo/ γ -Al₂O₃, is paramount for the optimization of the fuel conversion and the minimization of both resource consumption and production costs.

Despite their environmental benefits, nowadays bio-jet fuels remain significantly more expensive than conventional fossil-based aviation fuels.

The Minimum Selling Price of microalgae-derived biofuels has been estimated at 31.98 USD per gallon, making them substantially more expensive than conventional jet fuel [100]. To bridge this cost gap, financial incentives, carbon pricing mechanisms, and policy support are necessary. Governments and regulatory bodies can play a key role by implementing subsidies, tax credits, and mandates for sustainable aviation fuel blending. Additionally, co-product valorization (such as using residual biomass for bio-based chemicals or fertilizers) could help in lowering production costs and improving economic feasibility.

Aviation fuels must comply with strict safety and performance standards, making certification one of the hurdles for BJFs. ASTM and other regulatory bodies impose rigorous testing and approval procedures to ensure that biofuels meet specifications for energy content, viscosity, freezing point, and stability. Although

hydroprocessed renewable jet (HRJ) fuels have been certified under ASTM D7566, other pathways, such as those relying on genetically modified microalgae, still require further regulatory evaluation [99]. Moreover, the main barriers include demonstrating long-term compatibility with existing jet engines and fuel infrastructure [101]. To accelerate certification and adoption, as well as facilitate wider market introduction of BJFs, collaborations between researchers, industry stakeholders, and policymakers are essential.

9. Conclusions

This review analyzed the potential of photosynthetic microorganisms as a sustainable feedstock for bio-jet fuel production, focusing on the downstream processes from microalgal bio-oil extraction to fuel upgrading. The main bottleneck remains lipid extraction, which relies on costly solvents or energy-intensive techniques such as supercritical fluid extraction, hydrothermal liquefaction, and pyrolysis. Research is exploring cell disruption techniques and optimized process conditions to improve efficiency and reduce energy consumption. Microalgal bio-oil requires catalytic hydrotreatment to enhance energy content and reduce viscosity, yet high oxygen levels remain a challenge, affecting fuel performance. Optimizing deoxygenation, hydrocracking, and hydroisomerization processes is the key to producing aviation-compliant BJFs. Catalysts such as Pt/Al₂O₃, Rh/Al₂O₃, and NiMo/γ-Al₂O₃ play a crucial role in achieving the desired fuel properties, making catalyst selection and process optimization necessary for economic and technical feasibility.

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Conflict of interest

The authors declare that they have no conflict of interests.

Data availability

Data will be made available on request

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- 870

871 **Table 1.** Comparison between required characteristics for JF (ASTMD1655) and alternative JF (ASTMD7566), and characteristics of BD, BP, BHT, HBD, HBHT
872 (mean values and 95% confidence intervals). Elaborated from the dataset by Lutz et al [20].

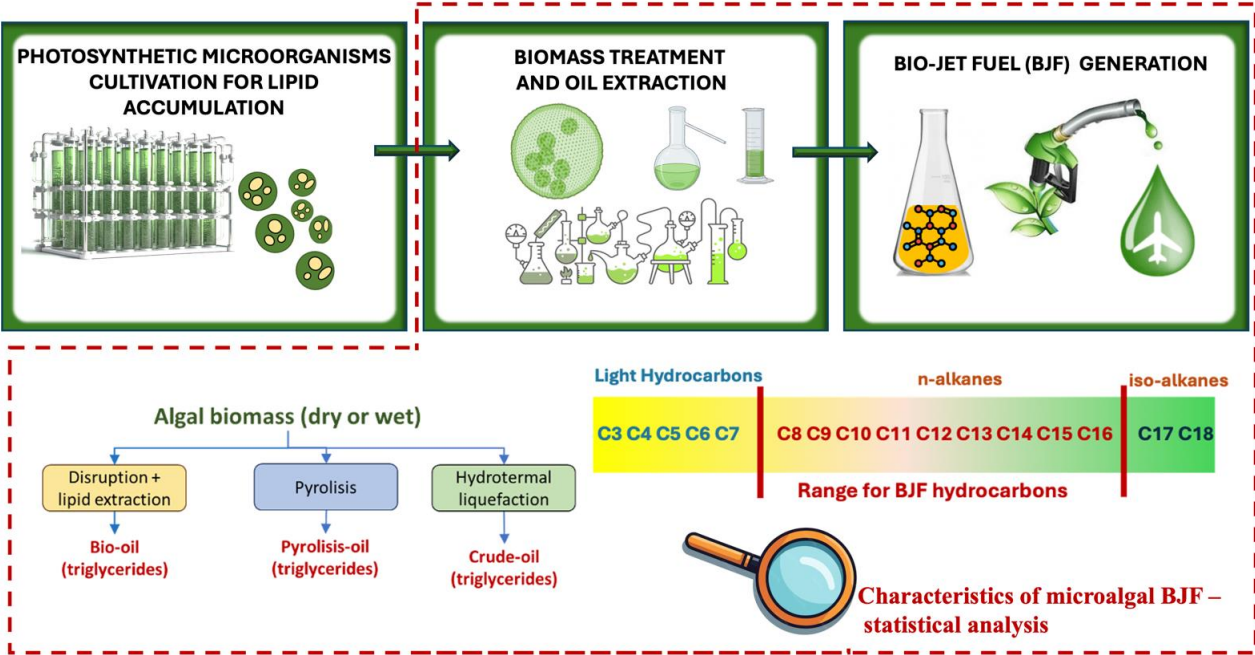
Property	ASTMD1655	ASTMD7566	BD	BP	BHT	HBD	HBHT
Acidity (mg _{KOH} g ⁻¹)	max 0.1	max 0.015	0.5 (0.2-0.8)	76 (54-98)	96 (0-217)		15 (6-24)
C and H (%w)	-	min 99.5	25 (8-43)	62 (56-68)	74 (67-81)	59 (18-100)	95 (94-96)
Sulfur (%w)	max 0.3	max 15 g kg ⁻¹	0.55 (0-1.1)	0.4 (0.3-0.5)	0.6 (0.5-0.7)		0.2 (0.1-0.3)
Water (%w)	-	max 0.75 ¹		22 (18-27)	5 (2-8)		0.4 (0.2-0.5)
ρ (kg m ⁻³)	775-840	730-772	908 (858-958)	1160 (1100-1219)	987 (909-1066)	833 (736-931)	841 (772-911)
T _F (°C)	max -40	max -40				-37 (-29-45)	
μ -20°C (mm ² s ⁻¹)	max 8	-	13 (3-23)	51 (13-89)	214 (89-340)	4.2 (1-10)	15 (7-231)
E (MJ kg ⁻¹)	min 42.8	-	38 (34-42)	26 (23-28)	36 (35-37)	44 (42-47)	43 (43-44)

873

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Graphic Abstract



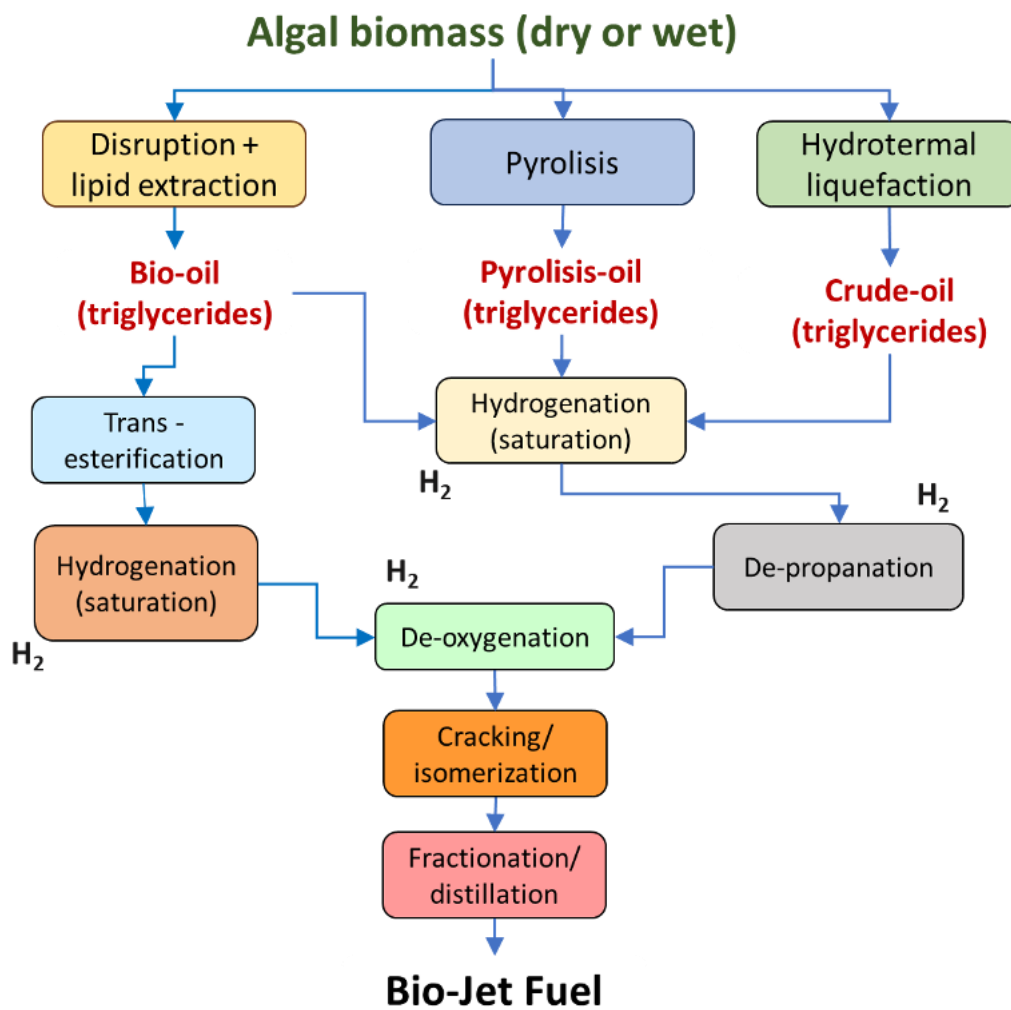
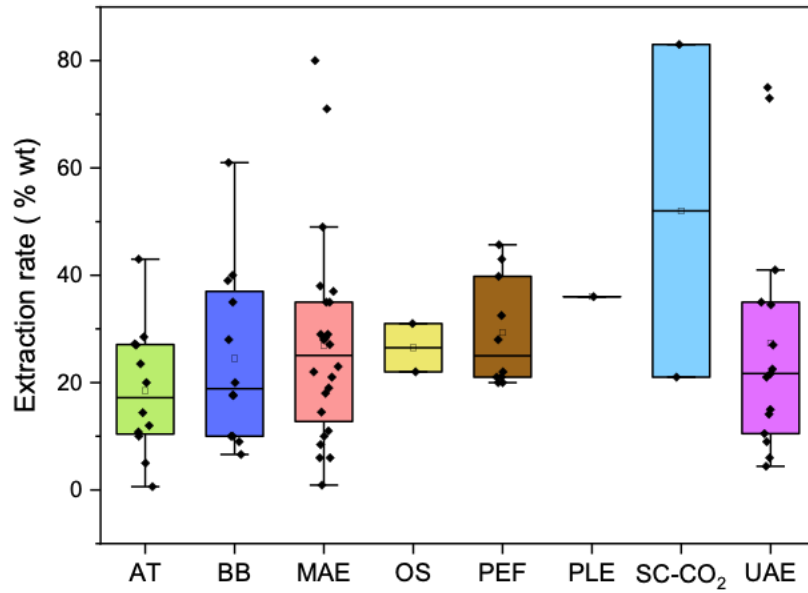


Figure 1. Main downstream processes for the production of BJFs from microalgal biomass.



AT = autoclave/thermal treatment, BB = bead beating, MAE = microwave assisted extraction, OS = osmotic shock, PEF = pulsed electric field, PLE = pressurized fluid extraction, SC-CO₂ = supercritical carbon dioxide, UAE = ultrasound assisted extraction

Figure 2. Extraction rates of lipids from microalgae using different techniques. Authors elaboration from the dataset reported by Lutz et al. (2024)

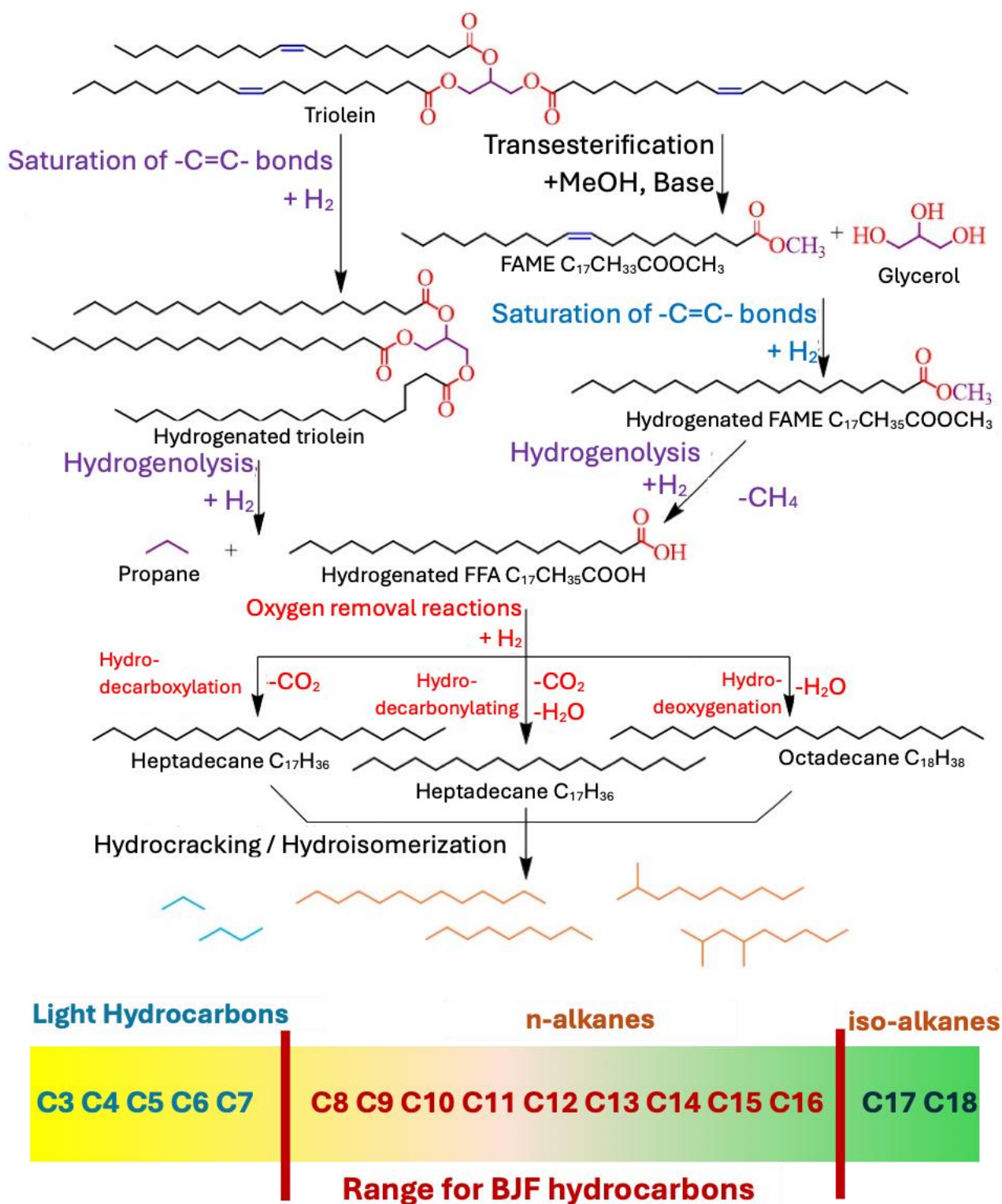


Figure 3. Conversion routes of extracted algal triglycerides (process shown using triolein) into BJT.

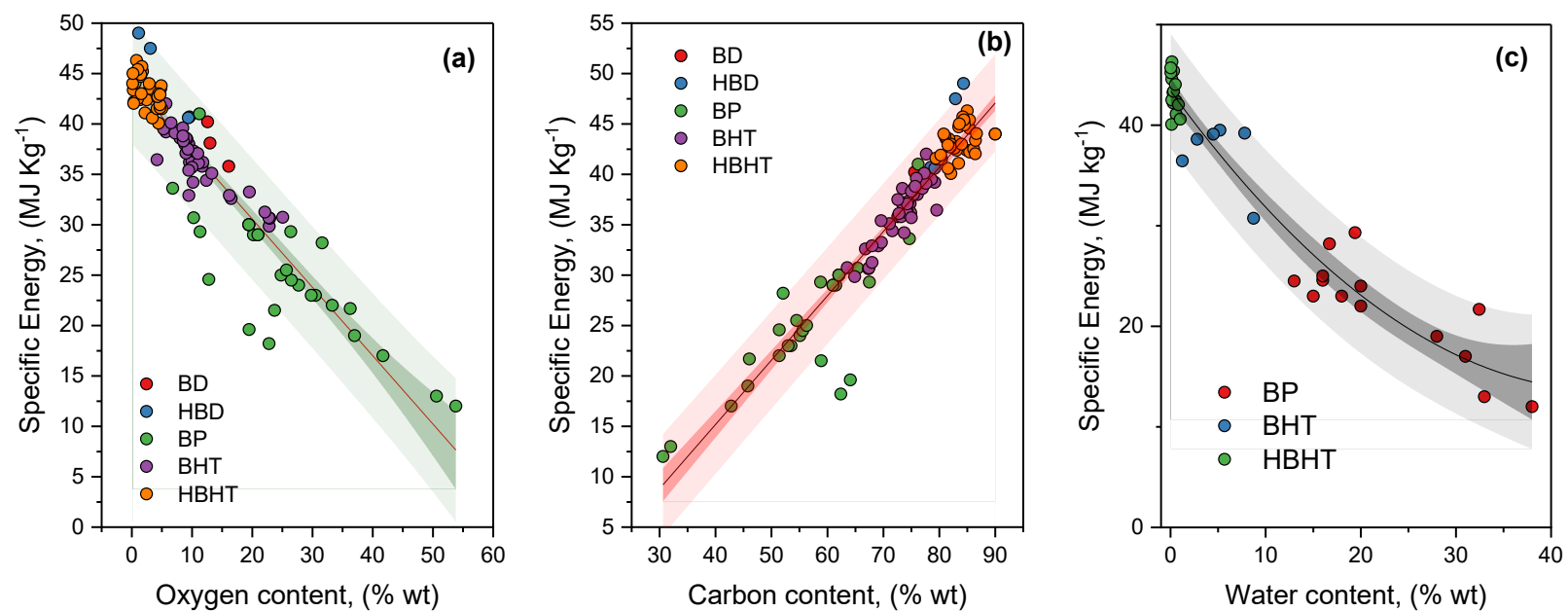


Figure 4. Correlations between Specific Energy of BJT and Oxygen (a), Carbon (b) and water (c) contents

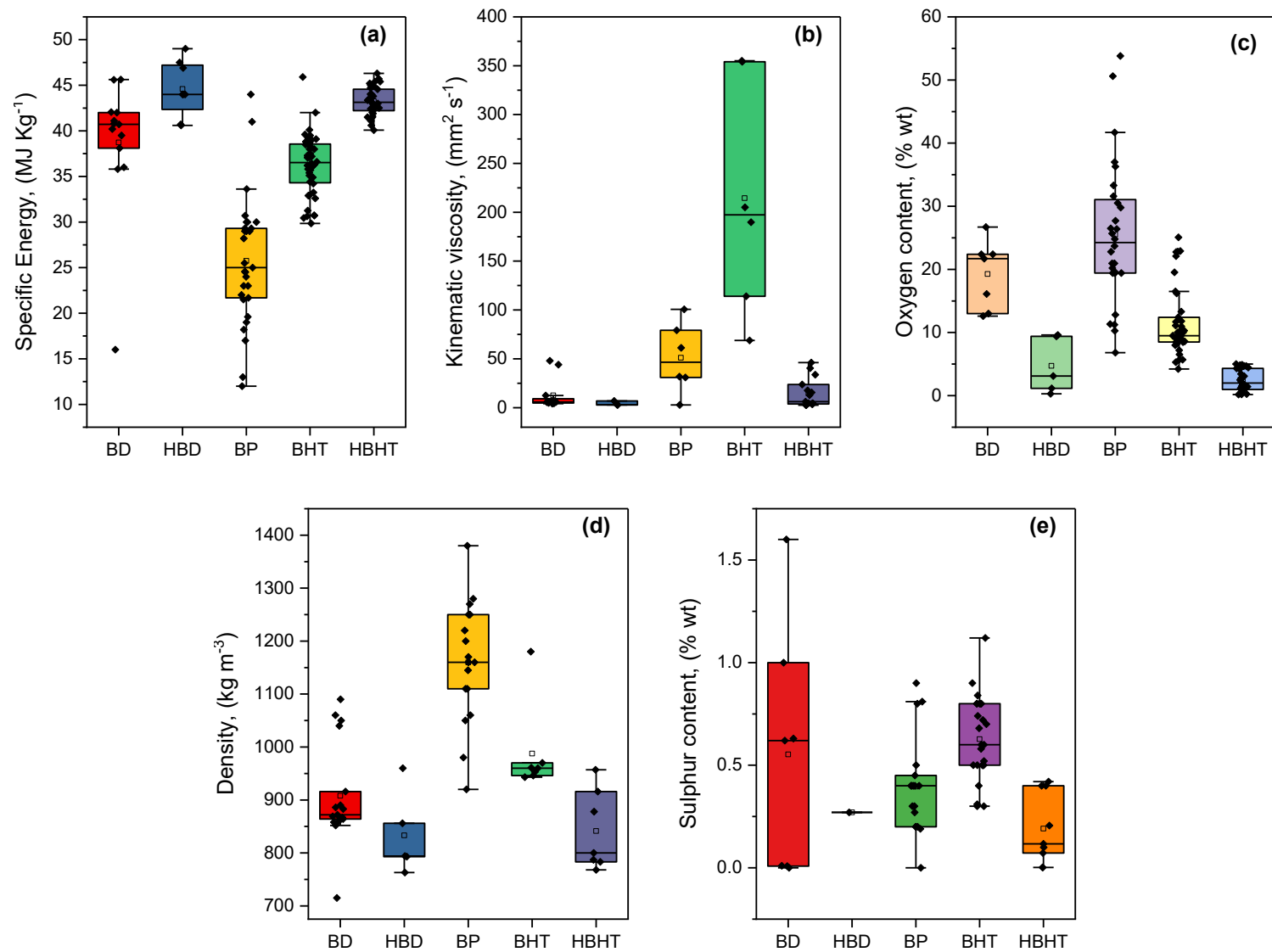


Figure 5. Box plot of some relevant characteristics of BJT obtained through the different techniques (a) Specific energy (b) Kinematic viscosity, (c) Oxygen content, (d) Density and (e) Sulphur content.