Journal of Environmental Chemical Engineering 12 (2024) 113886

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Engineering strategies of microalgal cultivation for potential jet fuel production – A critical review

Giovanni Antonio Lutzu [a], Luca Usai [a], Adriana Ciurli [b], Carolina Chiellini [c], Fabrizio Di Caprio [d], Francesca Pagnanelli [d], Ali Parsaeimehr [e], Ilze Malina [f], Kristaps Malins [f], Massimiliano Fabbricino [g], Alessandra Cesaro [g], Grazia Policastro [h], Giacomo Cao [i,j,k], Alessandro Concas [i,k,*]

[a] Teregroup Srl, via David Livingstone 37, Modena, MO 41122, Italy
[b] Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, Pisa, PI 56124, Italy
[c] Italian National Research Council, Institute of Agricultural Biology and Biotechnology (IBBA), Via Moruzzi 1, Pisa, PI 56124, Italy
[d] Department of Chemistry, University Sapienza of Rome, Piazzale Aldo Moro 5, Rome 00185, Italy
[e] Department of Agriculture and Natural Resources, College of Agriculture, Science and Technology, Delaware State University, Dover, DE 19901, USA
[f] Institute of Applied Chemistry, Riga Technical University, Paula Valdena Str. 3, Riga LV-1048, Latvia
[g] Department of Civil, Architectural and Environmental Engineering, University of Naples Federico II, Via Claudio 21, Naples, NA 80125, Italy
[h] Department of Engineering and Computer Science, Pegaso Telematic University, Centro Direzionale Isola F2, Naples, NA 80135, Italy
[i] Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, Via San Giorgio 12, Cagliari, CA 09124, Italy
[j] Center for Advanced Studies, Research and Development in Sardinia (CRS4), Loc. Piscina Manna, Building 1, Pula, CA 09050, Italy
[k] Department of Mechanical, Chemical and Materials Engineering, University of Cagliari, Piazza d'Armi, Cagliari, CA 09123, Italy

ARTICLE INFO

Keywords:
Aviation fuels
Bio-jet-fuels
Cultivation
Microalgae
Lipid accumulation strategies

ABSTRACT

The aviation industry stands as a well-known "hard to electrify" sector, currently responsible for the consumption of massive amounts of fossil fuels, considerably contributing to global CO_2 emissions. The need to reduce the aviation carbon footprint to comply with the European Green Deal objectives, has driven intensive research into the so-called bio-jet fuels (BJF) that can be obtained from different lipid-rich substrates, including microalgae among the most promising. Starting from the analysis of existing studies dealing with the selection of the strains more indicated for BJF production, this review examines the most recent breakthrough in microalgae cultivation techniques and lipid accumulation strategies, focusing on the approaches targeting the enhancement of the process environmental sustainability. The main bottlenecks in each phase of the production process are identified and critically reviewed. The most recent solutions are also thoroughly discussed to point out room for improvements in consolidated engineering strategies, as well as areas of further scientific research to advance the state of the art on micro-algal potential for BJF production.

1. Introduction

The aviation industry contributes for more than 2.5 % to the global anthropogenic greenhouse gas (GHG) emissions [1] and heavily relies on fossil fuels, accounting for 3 % of global oil consumption [2]. Finding alternative jet fuels (JF) is therefore crucial for the reduction of GHG emissions and for the transition to a new generation of fuels. To achieve this goal is very challenging due to the difficulty of electrifying the aviation sector and due to the limitations in the use of green hydrogen. An interesting alternative is based on the production of bio-jet fuels (BJF) using microalgal lipids. The process has been studied for more than 10 years, but still faces several constrains.

Moreover, the applied process to convert microalgae into BJF consists of several steps which are not yet optimized. These steps include lipid extraction, hydrothermal liquefaction, and pyrolysis followed by a bio-oil upgrade treatment consisting mainly of catalytic de-oxygenation, hydrocracking and hydroisomerization. Less often, BJF can be obtained from microalgae by gasification followed by Fischer-Tropsch or by sugar extraction followed by fermentation. Because of the mentioned constraints, microalgae-based technology for producing BJF is still not competitive and deserves further research and investments. To correctly address future studies, it is conducted an extensive review focusing on

* Corresponding author at: Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, Via San Giorgio 12, Cagliari, CA 09124, Italy.
E-mail address: alessandro.concas@unica.it (A. Concas).

https://doi.org/10.1016/j.jece.2024.113886
Received 24 May 2024; Received in revised form 13 August 2024; Accepted 18 August 2024
Available online 22 August 2024

the first steps of the process which, of course, affect all the downstream technologies in terms of applicability, efficiency, and economical convenience. The review starts from the analysis of existing studies dealing with the selection of the strains more indicated for BJF production, and then analyzes the factors influencing microalgae biomass and lipid production according to the most recent research conducted in this field. A particular emphasis is attributed to the new strategies proposed to enhance the process sustainability from both environmental and economical perspectives, including trophic conditions and the possible use of wastewater (WW). Finally, the most common full-scale cultivation systems are described and discussed, highlighting both design and operation aspects. The aim of the paper if to provide a clear idea of the progress in the upstream technologies of BJF production from microalgae. This will allow to better understand the existing limitations to be solved, and the possible opportunities to be explored and exploited in more details, to make the whole process available, so contrasting the noxious effects for the environmental quality coming from fossil fuel adoption in the aviation sector.

2. Strains used to produce BJF

The choice of microalgal species for BJF production is of utmost importance due to the distinct physiology of different strains, which can lead to the production of different molecules suitable for biofuel formulation. Microalgal lipids, which mainly consist of mono-, di- and tri- acylglycerols are typically used to produce, by transesterification, mixtures of fatty acid methyl esters (FAMEs), also known under the name of biodiesel. However, as reported in Table 1, biodiesel and jet-fuels (JFs) present significant differences and thus the former cannot be used in jet engine but is most suitable for road transportation.

It can be seen that JFs consist of mixtures of hydrocarbons (mainly alkanes) with carbon numbers ranging from 8 to 16 and a specific branching degree. In contrast, lipids, and thus FAMEs, from microalgae have mainly carbon chains ranging mainly from 14 to 22.

On the other hand, BJFs capable to mimic current JFs can be obtained by suitable catalytic upgrade of biodiesel (cf. Fig. 5). For this reason, the knowledge of FAMEs obtainable form microalgal bio-oils is of crucial importance to understand the possibility to produce BJFs from microalgae. The typical FAMEs composition of microalgae are reported in Table 2 and highlight that *Isochrysis galbana*, *Pavlova salina* and *Chaetoceros* sp. are among the strains producing higher amounts of myristic (14:0), palmitic (16:0) and palmitoleic (16:1) acids, while *Nannochloropsis*, *Tetraselmis*, *Scenedesmus* and *Chlorella* sp. are among the best producers of higher amounts of palmitic (16:0) and palmitoleic (16:1) acids. On the other hand, stearic, oleic and linoleic acids are mainly produced by strains *Dunaliella* sp., *Chlorella* sp., *Scenedesmus* sp. e *Tetraselmis* sp. It is not a case that the mentioned strains are those most frequently tested in the relatively limited existing studies, summarized in Table 3, specifically focused on the production of aviation fuel from microalgae cultivation.

Table 1
Main differences between biodiesel and JFs.

Parameter	Biodiesel	Jet Fuel (JF)
Chemical composition	FAMEs	Hydrocarbons (paraffins, naphtenes, romatics)
Use/ application	Diesel engine	Jet engine (turbine engine)
Energy density	Lower (around 37–40 MJ kg^{-1})	Higher (around 42–45 MJ kg^{-1})
Viscosity	Higher than JF	
Freezing point	Higher, which can be a limitation in cold climates	Lower, which is crucial for high-altitude flights
Carbon footprint (CF)	Lower than fossil diesel, renewable and biodegradable	Conventional JF has a high CF, but alternatives (i.e. BJF) are improving
Emissions	Lower CO_2, PM and Sox emissions than fossil fuels	Lower emissions with synthetic and BJF variants, but conventional JF has higher emissions
Storage stability	Prone to oxidation and degradation over tine	More stable over time
Regulatory standards	ASTM D6751 (USA), EN 14214 (EU)	ASTM D1655 (USA), DEF STAN 91–91, and others for aviation fuels
Compatibility	Can be used in existing diesel engines with little to no modification	Require compatibility with jet engine specifications and often blended with conventional JF

Table 3 reports, together with the tested strains, the main cultivation outputs, which allow to better understand either the convenience or the limitation related to each choice. As it can be easily deduced from Table 3, the freshwater strain *Chlorella* sp. NT8a exhibited the highest growth rates and, consequently, was characterized by a high biomass productivity [7,8]. Also the marine haptophyte *Pavlova salina* demonstrated a high growth rate [7,11], but while *Chlorella* sp. NT8a contained approximately 14 % of FAMEs, *Pavlova salina* had only 1.2 % FAMEs. Other marine strains, such as *Dunaliella salina*, *Tetraselmis suecica* and *Nannochloropsis* sp. strain BR2, had FAMEs contents exceeding 10 % [7, 11], even though their growth rates were not among the highest ones. According to other studies [12,13] cultivation of *Dunaliella salina* and *Chlorella* sp. allowed to reach an elevated percentages of total lipid production, with *Chlorella* sp. reaching about 40 % of dry weight and *Dunaniella salina* reaching 23 %. Unfortunately, in these studies the growth rate of the two strains did not result very high [12,13].

It is worth mentioning the study conducted by Bwapwa et al. [10] focused on *Nannochloropsis* sp., a prominent strain due to its unique biochemical and physiological characteristics, including high photoautotrophic biomass productivity and lipid accumulation, specific cellular xanthophyll pigments, lack of the chlorophyll *b* or c, and a high eicosapentaenoic acid (EPA) content. Another microalga, which resulted a suitable candidate for producing BJF, was *Schizochytrium* sp, tested by Kim et al. [14]. The authors succeeded to convert the produced lipids into 54.6 wt% polyunsaturated fatty acids (PUFAs) with a 87.7 % and 20.4 wt% purity of good quality BJF. Recently, also *Neochloris oleobundans,* was tested to produce an oil, which was compared with traditional JF, demonstrating its potential application in this field [15].

3. Biomass and Lipid productivity: possible strategies to enhance microalgal lipid content

As well known, lipid productivity (mass $volume^{-1}$ $time^{-1}$), can be calculated as the product of biomass productivity (mass $volume^{-1}$ $time^{-1}$) and the intracellular lipid content (%wt). In order to optimize lipid productivity it is therefore crucial to enhance the intracellular lipid content of microalgae, while maintaining high the biomass productivity [16]. Fig. 1 summarizes the data reported in the available literature related to biomass and lipid productivities, and to active growth and lipogenesis. According to the obtained trend, while there is a direct proportionality between biomass and lipid productivities, an inverse relationship exists between active growth and lipogenesis [17].

It follows that an optimal compromise between lipid content and biomass productivity must be determined, and a significant attention should be given to identifying the operating parameters and environmental factors that influence lipid accumulation in microalgae cells [18].

Apart from the specific physiology of different stains, which, as indicated in the previous paragraph, may affect lipid productivity, several cultivation methods can be adopted to enhance lipid biosynthesis of microalgae. All these methods share the common approach of subjecting microalgal cells to stress conditions, which trigger lipid synthesis.

Indeed, lipids, in the form of TAGs, serve as storage molecules that enable microalgae to withstand adverse environmental conditions by maintaining intracellular lipid homeostasis, cellular function and energy supply [17,31].

Table 2
Fatty acids (wt%) of microalgal strains used to produce biofuels [3–7].

Scientific name	C12:0	C13:0	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C18:4	C20:0	C20:1	C20:4	C22:0	C22:2	C22:6	C24:0
Nannochloropsis salina	5			0.5	37.5	23.3	0.4	0.9	11.9	1.5			0.1		3.3	0.4			
Nannochloropsis sp. BR2	0.2		3.5	0.4	33	26.8	0.4	3	6	0.9	0.4		0.2		5.4				
Nannochloropsis			1.27		11.24			2.22					4.33						
Nannochloropsis - HTL Process			4'741		36.44			1.45					1.5						
Phaeodactylum tricornutum			4.5		25.8	37.5		1.3		5.1	2				1.6				
Phaeodactylum tricornutum	8.94			12.06		4.89	15.82	3.18	2.47	0.67							0.61		
Thalassiosira sp.			6.37		20.67	42.02		0.27	0.7	1.82	1.52	2.51		0.1	0.22			0.8	
Nitzschia sp.	1.5		9		10.4	20.7		1.3	3.6	1.7	0.9	1.1							
Chaetoceros calcitrans			10.5		23.3	34.1	1.6	5.1	5.8	0.1	0				0.9				
Chaetoceros muelleri			11.6		26.2	29.7	1.8	4.5	1.7	0.2	0.4				1.4				
Botryococcus braunii	0.7		0.8	0.5	21	2	0.1	2.9	3.2	13.6	33		0.2			0.1			0.2
Ankistrodesmus sp.	3.1		4.7		13.1	3.4			8.6	5.4	28.6	9.8							
Dunaliella bardawil	5.6		7.1		46.2	0.9	0.7		17.2	7.9	8.1			1.2					
Dunaliella salina	4		5.4		14.7	0.7			2.2	10.9	30.5			1.2					
Dunaliella salina	0.1		0.6		24.7	2.9		5.8	5.6	7.6	33.8		0.1	0.1				0.4	
Dunaliella salina					4.44			1.45	24.71	18.77	50.65								
Dunaliella tertiolecta			0.47		17.7	0.88			4.87	12.37	30.19								
Tetraedron caudatum NT5	0.27		0.05		7.16	1.43		0.46	6.13	3.45	11.77		0	0	0	0.33			1.01
Chlorella sp.			1.62		16.46			3.27	14.64	20.61	15.35								
Chlorella sp.					4.45			0.51	34.9	20.56	39.33								
Chlorella sp. BR2	0.5		0.9	0.2	30.9	4.4	0.4	9.7	9.2	7.9	22.8		0.9	0.8	0.1				
Chlorella sp. NT8a	0.3		0.69		33.43	2.89		1.03	15.09	22.29	35.85		0	0	0	0			0
Scenedesmus dimorphus NT8c	0.22		0.18		22.21	1.9		1.59	24.45	6.29	17.71		0.31	0.33	0	0.32			0
Scenedesmus dimorphus NT8e	0.26		0.23		27.94	2.13		1.91	34.49	9.43	20.37		0.4	0.43	0	0.39			0
Scenedesmus dimorphus				5.6	21.3	4.2		2.8	23.4	5.8	24.3								
Scenedesmus obliquus			1.48		21.78	5.95		0.45	17.93	21.74	3.76	0.21							
Scenedesmus sp. NT1d	0.26		1.22		9.31	1.15		0.23	9.24	5.48	10.38		0	0	0	0.27			0
Tetraselmis sp.			1.22		17.45	3.27		0.3	17.91	5.95	16.97	2.24		1.34	1.26				
Tetraselmis sp. M8			0.4		22.5	1.1	4.5	3	9.1	11.7	28.9				3.4				
Tetraselmis sp. M8 - outdoor	0.8		4.2	0.5	20.8	1.3	2.5	10.1	13.6	7	11.1	12.7		4.6	0.1				
Tetraselmis chui	0.1		0.9	0.1	37.3	2.5	0.1	9	13.8	8.8	15.1		0.5	1.8	2.6				
Tetraselmis suecica	0.1		0.9	0.2	35.2	2.3		8.8	15.3	19.7	8.8		0.5	2.1	3.3				
Graesiella emersonii NT1e	0.25		0.09		18.79	2.39		2.04	23.72	11.04	18.36		0.2	0.29	0	0.23			0
Pavlova salina	0.2		19.4		24.8	3.6		8.3	2	1.1	1.3	6.1	0.4					10.5	
Pavlova lutheri			11.4		25	19.1		4.8	1.3		0.1			0.1	6.1			7.3	
Isochrysis galbana			19.2		16.4	2		4.4	21.7	0.7	3.1			5.9	13.9			11.8	
Isochrysis galbana				3.2	25.4	2.6		8.3	27.2	13.4	14.5								
Isochrysis sp.			13.3		11.7	6.3			15	3.7	5.6	16.6						12.8	
Spirulina			0.02		3.53			2.37											
Spirulina - HTL Process					6.69			1.18											
Spirulina maxima			0.34		40.16	9.19		1.18	5.43	17.89	18.32	0.08	0.06						

Table 3
Main strain used to produce bio-jet fuels and related cultivation outputs.

Strain (scientific name)	Cultivation mode	Operation mode	Growth medium	CO_2 conc (% vol)	Growth rate (day^{-1})	Biomass productivity ($g\ L^{-1}\ d^{-1}$)	Lipid content (%FAME wt^{-1})	Lipid content (% wt)	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)	Reference
Tetraselmis sp. M8	Flasks	Batch	Seawater + F medium	0.038	0.35	0.11 (0.08 outdoor)	2.5 (9.9 outdoor)	-	2.1 (4.8 outdoor)	[7]
Tetraselmis chui	Flasks	Batch	Seawater + F medium	0.038	0.35	0.06	3.2	-	1.5	[7]
Tetraselmis suecica	Flasks	Batch	Seawater + F medium	0.038	0.37	0.1	10.8	-	1.5	[7]
Nannochloropsis sp. BR2	Flasks	Batch	Seawater + F medium	0.038	0.32	0.08	10.6	-	6.2	[7]
Dunaliella salina	Flasks	Batch	Seawater + F medium	0.038	0.3	0.05	11.4	-	4.8	[7]
Chaetoceros calcitrans	Flasks	Batch	Seawater + F medium	0.038	0.34	-	-	-	3.2	[7]
Chaetoceros. muelleri	Flasks	Batch	Seawater + F medium	0.038	0.35	0.07	5.9	-	3.3	[7]
Pavlova salina	Flasks	Batch	Seawater + F medium	0.038	0.45	0.24	1.2	-	2.1	[7]
Pavlova lutheri	Flasks	Batch	Seawater + F medium	0.038	0.48	0.06	4	-	2	[7]
Isochrysis galbana	Flasks	Batch	Seawater + F medium	0.038	0.35	0.06	3.9	-	2	[7]
Chlorella sp. BR2	Flasks	Batch	Seawater + F medium	0.038	0.34	0.08	5.3	-	3.9	[7]
Chlorella sp. NT8a	Bottles	-	BBM	0.038	0.59	0.33	14	-	14.61	[8]
Scenedesmus dimorphus NT8c	Bottles	-	BBM	0.038	0.52	0.07	9.5	-	9.53	[8]
Scenedesmus dimorphus NT8e	Bottles	-	BBM	0.038	0.41	0.09	8.2	-	12.39	[8]
Tetraedron caudatum NT5	Bottles	-	BBM	0.038	0.37	0.02	6.5	-	2.71	[8]
Scenedesmus sp. NT1d	Bottles	-	BBM	0.038	0.48	0.03	6.08	-	3.17	[8]
Graesiella emersonii NT1e	Bottles	-	BBM	0.038	0.38	0.14	6.95	-	9.99	[8]
Chlorella sp.	Bottles	-	Conwy (modified)	0.038	-	0.09861	-	40.23	39.67	[9]
Dunaliella salina	Bottles	-	Conwy (modified)	0.038	-	0.07189	-	23.48	16.88	[9]
Spirulina	-	-	-	-	-	-	-	5.89 ± 0.11	-	[6]
Nannochloropsis	-	-	-	-	-	-	-	19.05 ± 0.13	-	[6]
Nannochloropsis	PBR	Batch	F/2 medium	-	-	0.18 (wet biomass)	-	-	60 (600 mg/L in 10 days)	[10]
Scenedesmus dimorphus	PBR	-	Bristol Medium	1.5	-	-	-	-	-	[3]
Isochrysis galbana	PBR	-	Erdschreiber's Medium	1.5	-	-	-	-	-	[3]

Accordingly, techniques involving the cultivation of algae under extreme pH and temperature conditions, high radiation, osmotic stress, and high heavy metals concentration, are currently under investigation [32,33]. While some of these methods have been demonstrated to significantly increase the lipid content of microalgae, most of them result in a significant reduction of the biomass growth rate, which, in turn, counteracts the positive effect of increased lipid content. On the other hand, prolonged stress can provoke the breakdown of the photosynthetic apparatus, resulting in chlorophyll degradation, inhibition of cell division, and overall reduction of productivity.

3.1. Light stress

Light is a physical parameter that can affect not only the performances of microalgal growth but also their lipid content. While a light intensity between 100 and 200 μmol $m^{-2}\ s^{-1}$ is commonly used for microalgal production, intensities in the range of 200–400 μmol $m^2\ s^{-1}$ can enhance the microalgae growth rate [34]. Gonçalves et al. [35] found that an increase of light intensity from 36 to 126 μmol $m^{-2}\ s^{-1}$ resulted in a substantial increase of lipid yield both for *Chlorella vulgaris* and for *Pseudokirchneriella subcapitata.* In the first case, the lipid yields passed from 4.6 to 28 mg g_{dw}^{-1}, while in the second case passed from 8.5 to 39 mg g_{dw}^{-1}. Similarly, *Scenedesmus abundans* exhibited an increase in lipid content from 21 % to 33 % when the light intensity was raised from 60 to 115 μmol $m^{-2}\ s^{-1}$ [36]. A very high lipid content, reaching 36 % of dry weight, was determined for *Botriococcus* sp. cultivated under 115 μmol $m^{-2}\ s^{-1}$ [36], while *Neochloris oleabundas* produced the highest lipid content (33 % of dry weight) under 280 μmol $m^2\ s^{-1}$ [37]. Higher light increase, from 105 to 415 μmol $m^{-2}\ s^{-1}$, almost doubled the lipid concentration in *Mychonastes homosphaera* which passed from 17.4 to 31.2 g L^{-1} [37]. Similarly, *C. sorokiniana, C. viscosa, C. emersonii, C. vulgaris, Pharachlorella beijerinckii*, and *P. kessleri* were able to increase their lipid productivity under a light intensity of 600 μmol $m^{-2}\ s^{-1}$ [38], while *Nannochloropsis* sp. experienced the accumulation of the highest amount of lipids (47 % of dry weight) under the light intensity of 700 μmol $m^{-2}\ s^{-1}$ [39].

In view of BJF production, it should also be considered that different values of light intensity can alter the composition of produced lipids,

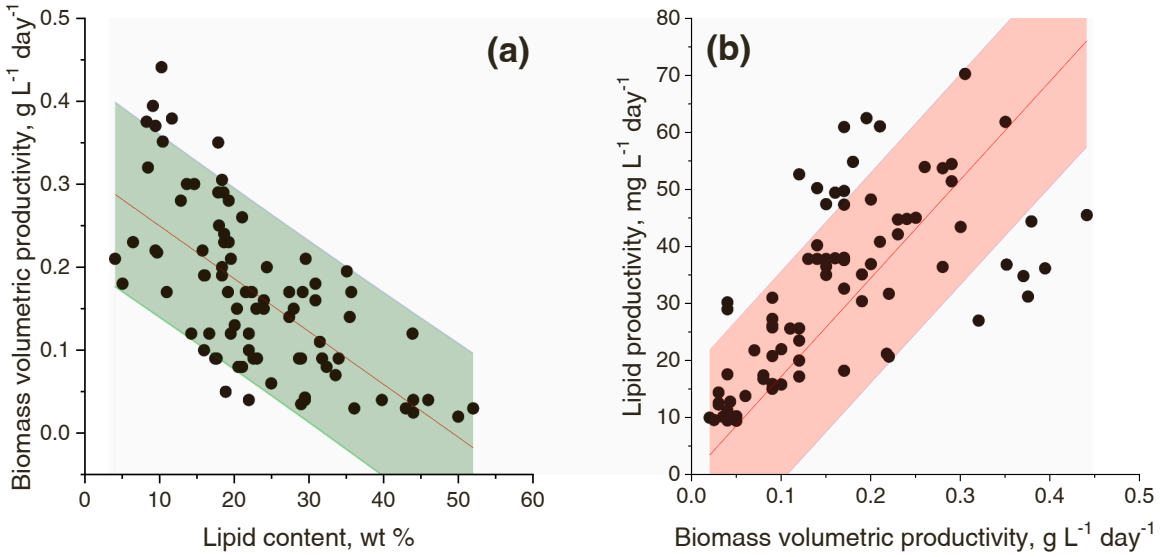

Fig. 1. Experimental correlation between biomass productivity and lipid content (a) and lipid productivity and biomass productivity (b) for several strains reported in the literature [19–30].

even when the overall total content of lipids remain unvaried. For example, PUFAs content of the red alga *Tichocarpus crinitus* increased under low light intensity whereas high light intensities resulted in greater accumulation of saturated fatty acids (SFAs) and mono-unsaturated fatty acids (MUFAs) [40]. Optimum light requirements are species-specific, and lipid productivity appears to be influenced by the light stress [41]. However, when the amount of provided light intensity is beyond the so-called saturation point, cells can experience photo-inhibition phenomena with damage to the photosynthetic pigments involved in the light capture process. To overcome this problem, a two-phase system can be applied during microalgae cultivation, cultivating the cells in the first phase under low light, and then shifting to higher light intensity.

3.2. Extreme temperatures

Temperature is another stress factor that significantly influences microalgal growth rate, net lipid productivity, and FAs profiles in a wide range of microalgal species [42,43]. Lipid production exponentially increases to a certain extent as the temperature increases and the optimal temperature value varies for different strains [44]. The optimum values of temperature to produce lipids was found to be around 20 ℃ for *Scenedesmus* sp. [45] and *Chlorella minutissima* [46], while it increased up to around 25 ℃ for *C. vulgaris* [47]. *Nannochloropsis oculata* showed an increase in lipid content from 8 % to 15 % as the temperature increased from 20 to 25 ℃ [47], while *S. obliquus* showed a lipid content varying from 18 % to 40 %, when the temperature increased from 20 to 27.5℃ [48]. Similarly to light, high temperature influences biofuel properties [43,49] and therefore has to be carefully controlled in view of producing BJF. Indeed, specific categories of lipids, mainly PUFAs, can decrease while increasing high temperatures. Wei et al. [50], for example, found a decrease in neutral lipids and PUFAs and a corresponding increase in SFAs and MUFAs production by *Tetraselmis subcordiformis* and *Nannochloropsis oculata* at increasing temperature. Luo et al. [49], instead, reported that unsaturated fatty acids (UFAs) levels increased at low temperatures, while total SFAs increased at high temperatures. The same authors also reported that *Chlamydomonas reinhardtii* showed a decrease in the total amount of stored FAs but an increase in the content of UFAs when cultivated at temperatures lower than 25℃.

3.3. Nutrient starvation

Nutrient starvation or limitation is considered the most effective approach to improve lipid content in microalgae and has been reported for most microalgal species [51]. This strategy is typically applied during a second phase of microalgal cultivation. A first phase consists of growing microalgae in media with sufficient nutrients to obtain high biomass concentration as quickly as possible. Later, when the cells population has increased, nutrient starvation is induced to promote the accumulation of lipids [52].

Generally, the limitation of P, in terms of phosphates, was found a stress factor causing an increase of lipid accumulation in almost all microalgal cells [53]. Mandal and Mallick [54], for example, reported a lipid content increase from 10 % to 29.5 % for *Scenedesmus obliquus*, limiting the amount of P made available to the microalgae. In turns, S starvation is found to trigger the accumulation of triacylglycerols (TAGs) in microalgal cells by diverting metabolic C-flow from protein to TAG synthesis [55]. However, the most widespread and effective technique to promote lipid accumulation is based on N starvation [56]. N is used to synthesize intracellular functional macromolecules (proteins) by combination with C obtained through photosynthesis. Under N starvation, the metabolic pathways leading to protein synthesis cannot be activated. As a result, while algal growth and replication are inhibited, the excess of internal C derived from photosynthesis is channelled into energy-storing molecules such as FAs, which are subsequently transformed into TAGs [57]. Fig. 2 summarizes the average effect of N starvation on lipid content in several microalgae strains, according to the data available in the scientific literature.

3.4. Strategies to identify the optimal stressing conditions

As discussed in the previous sub-paragraphs the lipid content is generally increased as a stress is produced on microalgae. On the other hand, this strategy can reduce the growth rate and thus the productivity of the culture. So, the challenge is to find the trade-off values of the stress-inducing operating conditions which boosts lipid synthesis while keeping unaltered the growth rate of microalgae. This goal could be pursued by means of suitable mathematical models that consider the relevant metabolic phenomena taking place within the cell and are capable to quantitatively evaluate the lipid productivity as a function of the operating variable which creates the stress. This way, the optimal values of the stress condition, i.e. the nitrogen concentration, light

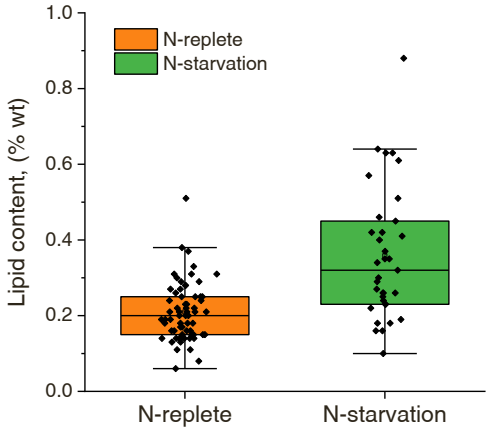

Fig. 2. Effect of nitrogen starvation on the lipid content of microalgae. Average values of several microalgae strains (>100) reported in Williams et al. [66] and Breuer et al. [67].

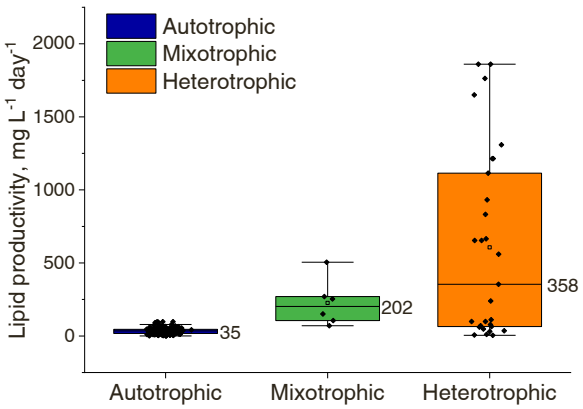

Fig. 3. Comparison among average lipid productivities obtained under the different metabolic regimes based on literature data in Table S1.

intensity, temperature, pH, salinity concentration, etc., could be identified and set. Starting from the pioneering work by Mairet et at., [58] which relies on the Droop cell quota concept, several mathematical models have been proposed in the literature to simulate the effect of nitrogen deprivation on growth rate and lipid production in *Isochrysis aff. Galbana* [58], *Coccomyxa Melkoniaani* [59], *Chlorella sorokiniana* [57] , and several others strains [60]. Also the effect of extreme pH, light intensity and iron concentration are also simulated by proper mathematical model presented in the literature [61]. Three relevant reviews have been so far presented in the literature regarding microalgae growth and lipid synthesis modelling [60,62,63] from which it can be inferred that, while the models so far proposed are capable to well grasp the experimental results obtained with specific strains under very specific lab conditions, no universally valid computational framework capable to evaluate "a priori" the optimal operating conditions under which lipid productivity is maximized is so far available. In this view the use of machine learning and artificial intelligence tools can play a crucial role and is starting to be explored in recent publications [64,65].

4. Microalgae cultivation conditions

As well known, microalgae can use C following either an autotrophic rout, or a heterotrophic one. In the first case inorganic C (CO_2) is assimilated via photosynthesis, and then reduced through the Calvin-Benson cycle. In the second case the C source is organic and is assimilated through oxidative phosphorylation.

Some microalgae can also exhibit a mixotrophic behavior, following a heterotrophic and a photosynthetic pathways, simultaneously [68]. Almost all studies aimed at verifying the potential of using microalgae to produce BJF (cf. Table 2) adopted autotrophic conditions during their cultivation. While this condition allowed the biofixation of CO_2 from the atmosphere or from flue gases, it is now known whether it was able to optimize lipid productivity. Indeed, as indicated in Fig. 3 and more detailed reported in the Supplementary Material (Table S1), heterotrophic metabolism may allow achieving a higher lipid productivity respect to autotrophic metabolism (up to three times higher), and mixotrophic metabolism may allow achieving a productivity similar to autotrophic metabolism. Therefore, cultivation conditions deserve a detailed analysis.

4.1. Heterotrophic metabolism

Heterotrophic nutrition takes place both in the presence and absence of light. In photo-heterotrophy, light act as an energy source whereas the sole source of energy during dark conditions is organic C. Cell growth and biosynthesis of products are significantly affected by medium nutrients and environmental factors. Genera reported to grow heterotrophically include *Amphora, Anabaena, Ankistrodesmus, Chlamydomonas, Chlorella, Chlorococcum, Crypthecodinium, Cyclotella, Dunaliella, Euglena, Nannochloropsis, Nitzschia, Ochromonas, Spirulina, Synechococcus,* and *Tetraselmis* [69]. Among the various strains listed in Table S1, *Chlorella protothecoides* [70] and *Euglena gracilis* [19] resulted the ones capable of providing the highest lipid productivities in heterotrophic conditions, close to 1300 mg L^{-1} day^{-1}. Heterotrophic microalgae might utilize C sources such as acetate, glucose, ethanol, glycerol, sucrose, lactose, galactose, mannose and fructose [71] coming from several substrates including waste materials. However, the presence of organic C easily exposes microalgae cultures to the detrimental impact of contamination by competing or predatory microorganisms. For this reason, heterotrophic growth media have to be absolutely sterile [71] or specific process control strategies should be applied to control biological contamination [72]. On the other hand, during respiration, CO_2 can be produced from organic C, making it possible for microalgae to couple heterotrophy with autotrophy in a two-phase cultivation strategy [73].

The comparison between heterotrophic and autotrophic growth has been studied on various microalgal species (Table S1). Compared to photo-autotrophic conditions, enhanced concentrations of biomass during heterotrophic conditions have been reported for: i) *Chlorella protothecoides,* up to 3.4 times [74]; ii) for *C. vulgaris*, up to 4.8 times [75]; and iii) for *C. sorokiniana*, up to 3.3 times [76]. The feasibility for large-scale biodiesel production based on heterotrophic cultivation of *Chlorella protothecoides* was outlined by Lee and Shen [77] and by Xiong et al. [78]. This microalga showed higher lipid content during heterotrophic growth [79], and exhibited a 55 % increase in lipid content when shifting from autotrophic to heterotrophic conditions [80]. Other studies also suggested a higher technical viability of heterotrophic production compared to photoautotrophic methods in either open ponds or closed photobioreactors for both *Crypthecodinium cohnii* [81] and *Galdieria sulphuraria* [82].

4.2. Mixotrophic metabolism

Only a few microalgae species can grow mixotrophically including the freshwater *Brachiomonas submarina*, *Chlorella* sp., *Chlamidomonas reinhardtii*, *Chlorococcum* sp., *Cyclotella cryptica*, *Euglena gracilis*, *Haematococcus pluvialis*, *Nannochloropsis* sp., *Navicula saprophila*, *Nitzschia* sp., *Ochromonas minima, Phaeodactylum tricornutum*, *Rhodomonas reticulata, Scenedesmus obliquus, Anabaena* sp., *Spirulina platensis* and *Synechococcus* sp. [83]. In mixotrophy microalgae simultaneously use inorganic CO_2 and organic C sources in the presence of light. Therefore, photoautotrophy and heterotrophy occur simultaneously. Photosynthesis, which is influenced by illumination, provides CO_2, while organic

compounds are assimilated through heterotrophic metabolism [84]. The ability of mixotrophs to process organic substrates means that cell growth is independent from photosynthesis, therefore light energy is not a limiting factor for growth [85]. Mixotrophy can be characterized by an increased microalgae growth and a better utilization of C sources by the cells with a photoautotrophic metabolism able to use some form of organic C. In this way, their shift towards autotrophy or heterotrophy depends on the culture conditions. During mixotrophic mode, acetyl-CoA in microalgae cells is generated from both the C source (i.e. CO_2 fixation obtained during Calvin cycle) and extracellular organic C. The possibility to simultaneously assimilate CO_2 and organic C may offer an opportunity to effectively cultivate microalgae with an efficient utilization of available light and organic C. It has been reported that mixotrophic metabolism has the ability to achieve four-time greater cells yields per unit of energy input when compared to autotrophy (0.00749 g cells kJ^{-1} vs 0.00177 g cells kJ^{-1}), which also implies a greater energy efficiency [86]. Lee et al. [87], for example, reported a maximum biomass concentration of 11.1 g L^{-1} in mixotrophic conditions with *Chlorella sorokiniana* compared to 2.2 g L^{-1} in photoautotrophic conditions.

So far, the lipid productivity in mixotrophic has not been investigated in detail. The few available data (cfr. Fig. 3), are indicative of a lower productivity respect to heterotrophic conditions. Nonetheless, it is worth mentioning that mixotrophic cultures exhibit reduced photoinhibition and improved growth rates over autotrophic and heterotrophic cultures. This is mostly due to the possibility to rely on both light and organic substrates to grow. The major limitations associated with mixotrophic cultivation are the simultaneous need for light, CO_2, organic C, and O_2 and a reduced energy conversion (i.e. from light to chemical energy) efficiency compared to heterotrophy condition, despite improved energy economics compared to autotrophic growth [86].

4.3. Wastewater to cultivate microalgae

Since the need to continuously supply nutrients can lead to high costs [88], the identification of cheap or costless sources of C and N has address the interest in the use of different kinds of wastewaters (WWs) for microalgae cultivation [89]. Many authors highlight the multifaceted role of microalgae through the achievement of a triple purpose: a) microalgae can aid in the decontamination of WWs, which are legally difficult to dispose due to their nutrients and pollutants threshold levels, without the need for expensive chemical-physical pre-treatments; b) microalgae cultivation reduces nutrients costs and brings ecological benefits; c) microalgae can integrate C fixation from atmospheric CO_2 and industrial emissions, leading to further environmental benefits in biodiesel production [88,90–92].

Municipal WWs are characterized by N and phosphate levels ranging between 15 and 90 mg L^{-1} and 5–20 mg L^{-1}, respectively, lower than those of agricultural WW [93]. The specific nutrient concentration (N, P and C) varies according to the treatment stage where WWs come from, and this may define the microalgal strain that can be grown without additional inputs. Dineshkumar et al. [94] treated municipal WW with the *Chlorella minutissima* strain, resulting in concomitant production of biomass and biofuel through the addition of CO_2. In a recent study, the same authors evaluated the possibility of recovering 68 % of chemical energy from algal biomass, with 44 % in bio-oil and 23 % in fuel gas [95].

Do et al. [96] focused on the possibility of using municipal WWs as a growth medium for 22 microalgae strains, with the main aim of targeting FAs for biofuel production. Only two strains, *Desmodesmus* sp. KNUA024 and *Pseudopediastrum* sp. KNUA039, were further analyzed for their FA composition and other properties relevant to biofuels. The results showed that *Desmodesmus* sp. KNUA024 had a high content of PUFA, particularly α-linolenic acid (54.83 %), whereas *Pseudopediastrum* sp. KNUA039 had a higher content of MUFA (41.36 %), including oleic acid (38.77 %). While most of the FAMEs from the microalgae complied with the ASTM 6751 and EN 14214 standards for biodiesel, only *Pseudopediastrum* sp. KNUA039 reached the required cetane number (CN) value (> 51) for biodiesel quality, mainly due to its high content of SFA and MUFA. The proximate analysis using TGA revealed that all the treated microalgae had relatively low moisture content and a high volatile content, making them efficient for combustion. This indicates that the strains used in the study were suitable for biofuels applications, including BJF, as they had appropriate moisture and higher volatile content compared to conventional bioenergy sources. In the study of Li et al. [97], *Chlorella* sp. was cultivated in a highly concentrated municipal WW and a FAME content in the dry biomass as high as 11.04 % was found. Notably, *Chlorella* sp. showed a high content of shorter chain FAs, primarily consisting of 16–18 C lengths, which are particularly advantageous for aviation fuel production.

The production of FAs by microalgal species was investigated also considering agricultural WWs as culture medium. Wang et al. [98] assessed the enhancement of the biomass productivity and accumulation of FAs by the *Chlorella pyrenoidosa*. The growth was compared in different dilutions of piggery WW to an artificial culture medium (Bristol's Medium). It was found that the lipid productivity was significantly higher in the former case, likely due to the lower biomass concentration in the Bristol solution. Notably, the most abundant FAs identified in cells grown with piggery WW were hexadecanoic acid (C16:0), linoleic acid (C18:2), and linolenic acid (C18:3), which all approached around 30 % of the lipid content. The relative content of linolenic acid (C18:3) was about 27 % (w w^{-1}), far higher than the limit (about 12 %) established by the European Standard EN 14214, thus highlighting the need for the process optimization. Interesting results were obtained also by Johnson and Wen [99] who focused on the growth of *Chlorella* sp. algae using dairy WW as growth medium. This study compares the biomass growth of algae attached to different supporting materials to algae suspended in medium. The attached culture showed higher biomass production compared to the suspended culture. Among the tested supporting materials for algae attachment, polystyrene foam showed strong attachment, resulting in high biomass yield (25.65 g m^{-2}, dry basis), and high FA yield (2.31 g m^{-2}). After a 10-day regrowth culture, the attached culture system demonstrated a high potential for biodiesel production with FAMEs yield of 2.59 g m^{-2} and a productivity of 0.26 g m^{-2} day^{-1}. Moreover, the algae contained several major FAs, including C16:0, palmitoleic acid (C16:1), stearic acid (C18:0), C18:1, and C18:2, with percentages of 20.3 %, 7.35 %, 16.29 %, 32.28 %, and 8.34 %, respectively. The total fatty acid (TFA) content varied from 8.0 % to 10.7 % of the dry biomass.

Industrial wastewater (IWW) has also gained attention and most studies on the microalgal growth in IWW considered *Scenedesmus* sp. Hodaifa et al. [100] used olive-oil extraction WWs that tend to be low in N. Although reduced N content can inhibit the exponential growth resulting in low biomass productivity, this N deficiency enables certain microalgal species to accumulate PUFA, which are essential from a commercial point of view. In fact, the studied species *Scenedesmus obliquus* presented a limitation in biomass production, even though it showed a high content of MUFA (25.2 % and 45.3 %) and PUFA (16.6 % and 23.1 %) when using 50 % and 100 % of rinse water (RW) from oil-olive extraction, respectively. In particular, using RW as a complete culture medium, FAs values such as 24.2 % for C16:0, 39.4 % for C18:1n-9, and 12 % for C18:2n-6 were obtained.

In a previous study [101], *Scenedesmus dimorphus* growth was evaluated using brewery WW as a culture medium and biomass production as high as 6.82 g L^{-1} was obtained, along with a lipid accumulation up to 44.26 % DW. The FA profile of the microalga showed that 93.47 % of its composition consisted of FAMEs. The FAME profile of *S. dimorphus* cultivated in brewery WW indicated favorable conditions in terms of oxidative stability, ignition quality (cetane number), cloud point, melting point, and lubricity, which are crucial for meeting biodiesel quality standards set by both ASTM and European and Chinese National

Standards.

The existing literature show that different kinds of WW can be purified by microalgae, which are able to utilise the WW pollutant load for their growth. In most cases, the microalgal biomass grown on WW has a composition suitable for the generation of aviation fuels. Nonetheless, additional studies should be carried out to further investigate the possible inhibition of microalgae from the presence of specific contaminants that can be found in WW, with the main aim of widening the variety of strains to be cultivated on WWs and to optimize the process in the view of the aviation fuel generation.

5. Microalgae cultivation systems – engineering aspects

The engineering of the process, including the geometric configuration of the reactor and the operation regime, greatly influences its proper development and the microalgal biomass and lipid productivity. The choice of photobioreactor (PBR) geometry can significantly impact the quantity and quality of microalgal biomass. Closed systems can provide precise control over cultivation parameters, preventing contamination more effectively than open systems. On the other hand, open systems are advantageous due to their simplicity, lower construction, and operational costs, making them more accessible. When low-cost biomass for fuel production is the main objective, open systems may be a suitable choice [102]. Concerning the feeding mode, the main alternatives are batch, continuous, and semi-continuous operation. Batch and continuous systems have several advantages, such as high biomass productivity and low operational costs. However, the semi-continuous mode can easily achieve high lipid content due to environmental stresses [42]. Reactors can be operated both in single stage and double stage. While double stage reactors allow for a higher lipid accumulation, they are more expensive compared to single stage ones. The overview of the main engineering aspects related to microalgae cultivation is schematically reported in Fig. 4.

5.1. Cultivation of algae in open ponds

In open systems, the ponds are exposed to the environment, offering the advantages of using natural resources without cost. At a large scale, various designs for open ponds, whether natural or artificial, have been suggested. Among these designs, the most common include unstirred ponds (such as lakes and natural ponds), inclined ponds, central pivot ponds, and raceway ponds, based on their mixing and cultivation methods [26]. Currently, over 80 % of algal biomass is produced in open ponds, primarily due to the lower initial investment.

The "raceway pond" is the prevalent form of open pond and consists of a series of open channels. In these channels, a paddlewheel is employed to drive the flow while keeping algae suspended in the water along a racetrack. The channels are also equipped with baffles to guide the flow and optimize space utilization. The ponds are generally operated continuously with a fresh medium containing macro and micronutrients introduced in front of the paddlewheel, while the algal broth is harvested behind it after circulating through the loop [103]. Typically, raceways are constructed using concrete, but they can also be created by digging into the soil and lined with plastic to prevent leakage into the ground [42]. A notable characteristic of raceways is the shallow water depths, usually around 15–20 cm, to ensure sufficient light penetration throughout the hydraulic section, thus preventing dark zones where microalgae cannot thrive. With such depths, biomass concentrations of around 1 g L^{-1} can be achieved, and productivities ranging from 15 to 25 g m^{-2} day^{-1} are possible [104].

Open ponds are currently regarded as the most economical method for the large-scale production of microalgae [105]. However, they present several limitations compared to closed systems. Firstly, a significant drawback is their lower productivity when compared to closed systems. This is mainly due to changes in the ionic composition of the growth medium caused by evaporative losses, leading to potential problems such as hyper-salinity and nutrient precipitation. Moreover, open ponds cannot effectively control changes in temperature and photoperiod due to seasonal variations [106]. Since sunlight serves as the primary light source for photosynthesis, only the top layer of the pond (usually a few centimetres deep) is exposed to sufficient light, while deeper parts may experience limitations in light availability. The C source, typically atmospheric CO_2, has a very low transfer rate, potentially leading to C starvation and even O_2 produced by photosynthesis has a low transfer rate, that can generate excessive O_2 accumulation in the medium. To mitigate the drawbacks related to CO_2 and light limitation, improving mixing and introducing air bubbling at the bottom of the ponds using appropriate spargers can help. Overall, the low productivity of these

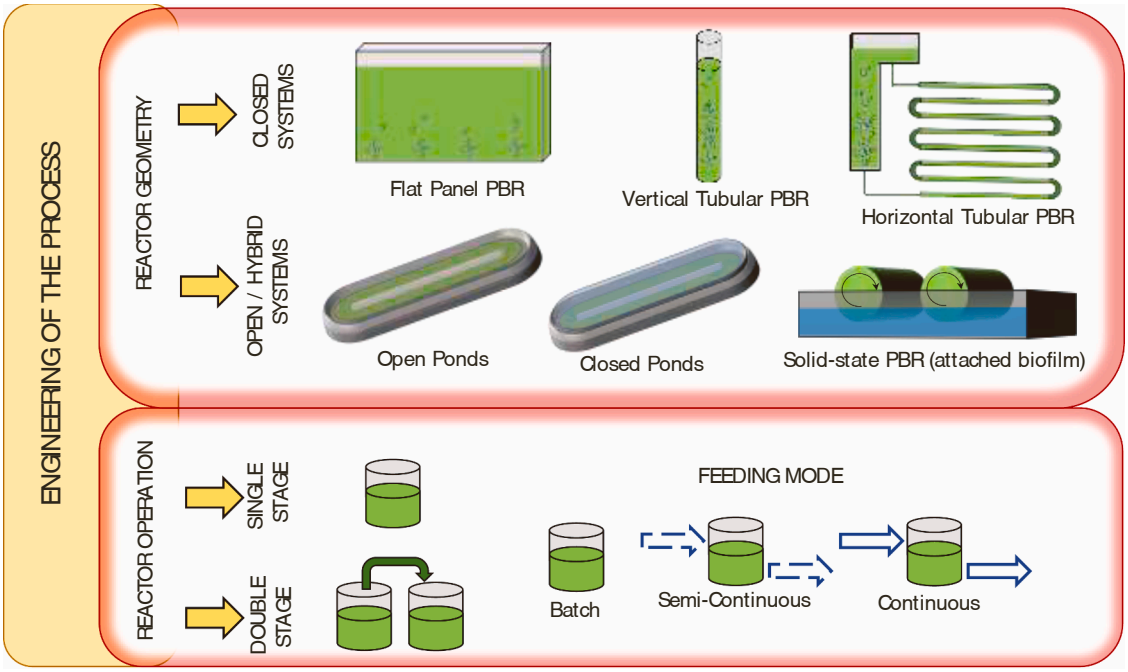

Fig. 4. Overview of the engineering aspects of the microalgae cultivation process, reporting the main alternatives related to reactor geometry and operation.

open pond systems may necessitate significant land areas to achieve the desired cultivation output [28].

5.2. Cultivation of algae in closed systems

PBRs are closed systems with no direct exchange of gases and contaminants with the environment. They expose culture broth and microalgae to higher photon energy flux than open ponds, promoting photosynthetic processes and biomass growth, due to their higher surface to volume ratio. PBRs offer better control and optimization of critical operating parameters such as temperature, pH, nutrient concentration, light intensity distribution, mixing, and gas mass transfer rate. Consequently, PBRs typically have higher biomass productivities than open ponds. However, PBRs are more expensive and complex to operate compared to open ponds [26].

The choice of PBRs depends on several factors, including desired productivity of microalgal biomass and the final products. While closed PBRs offer benefits such as better control and reduced contamination issues compared to open systems, they require additional costs for providing light illumination, CO_2, and cultivation feedings. Despite these additional costs, closed PBRs are still considered simpler to control. However, closed PBRs also have some limitations, such as the formation of biofilms, which can lead to oxygen accumulation in the culture with potentially toxic effects on photosynthetic growth. Additionally, light availability remains a primary limiting factor for microalgal growth even in closed systems. When constructing a PBR, several key criteria need to be considered, including the role of light, circulation, mass transfer, the choice of construction materials, and temperature control. These factors play a crucial role in ensuring the efficiency and success of the microalgal cultivation process in closed systems. An ideal PBR design should efficiently capture all available sunlight and uniformly distribute it in the growth medium where algae are suspended, enabling optimal light utilization for biomass formation. This necessitates a critical design parameter known as the illumination surface area per unit volume, with higher values leading to greater volumetric productivities [107].

PBRs come in different configurations such as tubular, column, membrane, and flat panel. Tubular PBRs are mainly used for outdoor mass cultivation and are typically made of glass or plastic. They can be arranged in different orientations, such as horizontal, inclined, vertical, or helical, to maximize sunlight capture. This orientation optimizes light harvesting and can be further enhanced by covering the ground with white plastic sheets. The microalgal culture is pumped into these PBRs, which generally have diameters ranging from 10 to 60 mm and length that can reach up to 100 m. A smaller diameter of 10 mm may be chosen for the high cell concentration to ensure suitable light penetration, while larger diameters may be used with proper fluid turbulence to promote algae movement between illuminated and dark zones. Despite their ability to capture light effectively, horizontal PBRs may face challenges such as photo-inhibition and heat accumulation, necessitating expensive temperature control systems like heat-exchangers. Tubes fouling due to cell adherence can also impact light penetration in the culture [108]. In outdoor cultivations, they often suffer from limitation in the photosynthetic efficiency due to O_2 build up with significant energy consumption compared to bubble column and flat plate PBRs. Moreover, due to the photo-limitation and mass transfer problems, the growth of the cells in the center of the tube is reduced. To address these issues, the width of the tubes should ideally be kept as short as possible, taking into account potential O_2 accumulation and CO_2 limitation. Another drawback of these systems is the uncontrolled grow of pathogenic microorganisms on the inner walls and the formation of biofilms, which influence the mass transfer of reagents. It has been demonstrated that the presence of external mass transfer resistance at the biofilm surface can create concentration profile switches inside the biofilm.

Among different PBR types, the bubble column is a classical configuration of vertical tubular PBR. It is a simple cylinder device with a height-to-diameter ratio kept greater than 2 to maximize the surface area-to-volume ratio, with a recommended radius not exceeding 20 cm to avoid issues related to light availability at the center of PBR. Additionally, the height is usually limited to about 4 m due to structural considerations, including the strength of the transparent materials used and to prevent shading effects [28]. CO_2 is supplied to the algae by bubbling gas from the bottom, promoting mixing without causing significant shear stresses on microalgae. Additionally, the gas flow aids in the efficient removal of photosynthetic O_2, preventing its accumulation, which could inhibit growth. The PBRs offer advantages such as low shear forces, absence of wall growth, and high mass transfer, resulting in efficient utilization of CO_2. The efficiency and their maximum biomass productivity depend on various factors, including the column dimensions, specific growth rate or doubling time of the algae strains, light intensity, and surface area. Cell density affects light penetration due to mutual shading effects between different cells. Therefore, to prevent the sedimentation of microalgal cells, proper mixing through aeration is necessary. Aeration ensures uniform exposure to light and nutrients, facilitates heat transfer, and promotes gas exchange. However, the size of the bubbles is influenced by various factors, including the properties of sparger, physical properties of the liquid and gas phases, and the column's H/D ratio. Additionally, considerations need to be given to phenomena like bubble coalescence or breakage and the possibility of clogging effects caused by the presence of micron-sized algae, which is more common at higher cell densities.

Flat panels are cuboidal-shaped PBRs, with minimal light path and a large illumination surface area (SVR). They can be made from transparent materials like glass, plexiglass, or polycarbonate. In these systems, CO_2 is provided by bubbling gas from one side through suitable perforated tubes. These PBRs offer several advantages, including a high ratio of illuminated area to volume, easy temperature control by spraying water onto the irradiated surface or submerging the bottom of the PBR in a water pool, low mechanical forces on the cells, high gas-liquid mass transfer rate, and efficient mixing provided by air bubbling or mechanical rotation. Due to these benefits, these PBRs are emerging as particularly suitable for mass cultivation of photosynthetic microorganisms [109]. However, conventional flat panels have challenges in controlling liquid flow and higher construction costs. Vertical alveolar panels made from plexiglass have been proposed as an alternative, offering a high surface-to-volume ratio (about 80 m^{-1}) and improved mixing with lower manufacturing costs [110].

A significant limitation of PBRs is the risk of biomass wash-out due to short residence time, leading to a harvesting rate that exceeds the growth rate. This aspect can be solved by membrane PBRs (MPBRs). In these systems, an additional filtration tank with a membrane retains microalgal cells, preventing wash-out and increasing biomass concentration, while the medium passes through as permeate. MPBRs can be operated at higher dilution and growth rates compared to PBRs [111]. However, one key challenge for large-scale cultures is the recycling of remaining nutrients in the permeate to minimize water and nutrients consumption.

Recently, plastic bag PBRs have gained attention due to their low cost. These bags can be arranged in different patterns based on their volume while offering versatility as they can be immersed in a water pool to control temperature in summer or even designed for cultivation in the ocean, utilizing ocean waves for mixing and mass transfer to substantially reduce cost [109]. However, plastic bag PBRs have disadvantages, including inadequate mixing, leading to uneven cell growth, and photo-limitation due to the bag's shape. They are also fragile and prone to leakages, resulting in a short lifespan (usually a few months).

Solid-state photobioreactors (PBRs) or attached cultivation is the last frontier of cultivation. These systems represent an advanced method for cultivating microalgae, differing from traditional liquid-based systems by enabling algae to grow on solid or semi-solid surfaces. They rely on the natural ability of microalgae to form biofilms (that is complex

communities of microorganisms attached to surfaces), allowing them to capture more efficiently light and nutrients from the environment [112]. They have been studied to improve productivity and reduce water usage, and are particularly advantageous in scenarios where water conservation, space efficiency, and optimized light utilization are crucial. In these cultivation systems, algal cells are immobilized on substrates like membranes, fibers, meshes, or specially designed surfaces (made from materials such as glass, plastic, or even natural fibers), allowing them to grow while a minimal volume of culture medium continuously supplies nutrients [113]. Common designs for attached cultivation systems include rotating discs, vertical panels, and inclined surfaces. In rotating disk systems microalgae grow on the surface of discs partially submerged in the culture medium. As the discs rotate, algae are alternately exposed to light and nutrients, optimizing growth. In vertical panels algae are grown on vertical surfaces that are positioned to maximize light capture. This setup is especially useful for outdoor operations where sunlight is the primary energy source. Inclined surface systems involve algae growing on sloped surfaces, where the culture medium flows over the algae, providing nutrients and preventing desiccation. Solid-state PBRs are particularly useful to treat WW by removing nutrients such as N and P. The high surface area-to-volume ratio in these systems facilitates the production of biomass that can be converted into biofuels and valuable compounds like pigments, antioxidants, and omega-3 fatty acids for use in pharmaceuticals, cosmetics, and food supplements [114]. This system requires significantly less water than traditional PBRs because the algae are not fully immersed in a liquid medium. Nutrient use is also more efficient as they are directly delivered to the algae on the substrate. These systems operate by circulating a culture medium over or through substrates where microalgae are attached. Light penetrates the biofilm layer, enabling the microalgae to perform photosynthesis, converting CO_2 and water into oxygen and biomass. The biofilm structure supports a high cell density, which improves light capture and nutrient use efficiency. In certain designs, air or CO_2-enriched gas is bubbled through the PBR to enhance gas exchange and prevent the biofilm from becoming too thick, which could block light and decrease photosynthetic efficiency. By utilizing vertical or inclined surfaces, these systems can achieve high biomass densities in a smaller footprint, making them suitable for both urban and rural environment. At a lab scale, attached cultivation systems have demonstrated the highest biomass productivities and easiest harvesting procedures with high gas exchange rates when scaled up. Biomass productivity in attached cultivation PBRs can be substantially higher than in traditional suspended systems, with reported yields ranging from 20 to 40 g m^2 day^{-1}. This productivity depends on factors such as light intensity, nutrient availability, and the specific type of microalgae used [115,116]. These rates are often two to three times greater than those in suspended systems under similar conditions, making biofilm PBRs particularly appealing for high-value applications like biofuel production or nutraceuticals. Multiple layers can be arranged to increase the illuminated area and promote the photo-induction of algal cells [117]. This approach has shown success in both indoor and outdoor operations, with several freshwater and marine species exhibiting high biomass productivities and photosynthetic efficiencies. On the other hand, there are also some drawbacks related to biofilm formation, complex harvesting and their maintenance and scale-up. As microalgae grow, they can form dense biofilms on the substrate, which may hinder light penetration and nutrient uptake, reducing overall efficiency. The systems require regular cleaning to prevent fouling of the substrate, which can be labour-intensive and costly [118]. Harvesting algae from solid surfaces can be more challenging than from liquid cultures, often requiring specialized equipment or manual labour. While highly efficient in small-scale operations, scaling up solid-state PBRs can present technical challenges, particularly in maintaining uniform light exposure and nutrient delivery [119]. Even though biomass production costs are higher compared with the suspended cultures, attached cultivation necessitate the need to increase productivities and light efficiencies while finding the best balance between production cost and biomass yield, composition, and productivities.

To address the limitations of both open and closed systems, closed ponds are being explored as a compromise [120]. This solution involves covering the open pond with a transparent or translucent barrier to create a greenhouse effect. The working principle of this configuration entails circulating microalgal cultures through a looped channel using a paddlewheel or pump, ensuring continuous motion. The system is enclosed to prevent contamination and water loss while allowing light to penetrate. CO_2 is often injected to enhance photosynthesis, and nutrients are supplied in a controlled manner, enabling better control of environmental conditions like temperature, light, and pH. Additionally, using closed raceways allows for increased CO_2 supply since the gas, when bubbled from the bottom, cannot escape to the atmosphere [103]. Among their advantages higher biomass productivities, efficient CO_2 capture, and moderate energy consumption can be listed. Biomass productivity in these systems (potentially increased up to 30 g m^2 day^{-1} under optimal conditions) falls between those of open raceways and fully closed PBRs, making them ideal for biofuel production where controlled stress conditions can increase lipid content [121]. CO_2 fixation rates of 1.5–2.0 g CO_2 m^2 h^{-1} and energy consumption at 1.0–2.0 kWh kg^{-1} of biomass make them suitable for integration with industrial processes [19]. However, they have higher initial costs, require more maintenance, and face challenges in light distribution and scalability, particularly in maintaining uniform conditions in large-scale operations [122]. Despite these challenges, closed raceway systems hold significant potential for large-scale biofuel production and environmental applications, allowing to maintain a similar cost-effectiveness compared to open systems.

5.3. Operation modes

As mentioned earlier, the choice of microalgae species and the type of cultivation system play crucial roles in determining the productivity and success of microalgae biomass production. The development of robust and efficient cultivation strategies is essential for maximizing biomass yields and ensuring the viability of microalgae-based industries. Overall, the chosen operation strategy is vital for commercial feasibility in microalgae-based biofuel production.

Batch operation boosts microalgae growth and product concentration due to nutrient flexibility. However, the limited light availability at high cell density negatively affects biomass productivity. One proposed solution could involve a stepwise increase in light intensity, but this strategy is challenging to control, especially in outdoor cultivation with fluctuating light [42]. To reduce self-shading effects, continuous cultivation is an appealing option for microalgae growth. It maintains high biomass productivity by continuously feeding medium and nutrients, while continuously extracting the effluent for large-scale industrial applications. However, maintaining steady-state conditions may hinder lipid accumulation, as nutrient-rich and non-stressful environments prevail. Adjusting the dilution rate and using artificial light sources could help achieve stable continuous culture, overcoming potential challenges of slow growth and washout during night periods [123].

The semi-continuous operation is a highly efficient bioreactor strategy in microalgae-based biofuel production. It maintains exponential growth conditions, avoiding low cell division rate and light limitation. Semi-continuous processes are practical for long-term cultivation and suitable for industrial production of microalgae-derived lipids. Studies have shown significant enhancements in biomass productivity and lipid content using this approach. Yadav et al. [124] reported increased biomass productivity using the semi-continuous regime applied to outdoor raceway ponds, which resulted in a biomass density of 0.42 g L^{-1} and 3.5-fold increase in areal productivity (11.49 g m^2) compared to the batch system. While this system appears promising, its feasibility for large-scale outdoor operations requires further evaluation [42].

Previous studies have also evaluated the possibility of conducting

two-stage processes. The two-stage strategy involves a first stage that employs nutrient-rich medium to maximize biomass productivity. Once sufficient microalgal biomass is obtained, stress conditions are induced in the second stage, to boost lipid accumulation [125]. San Pedro et al. [126] proposed a continuous operation process for the first stage to enhance biomass via dilution rate adjustment. In the second stage, various stresses were applied to boost lipid accumulation, while maintaining reasonable biomass productivity. This system resulted in higher lipid productivity compared to the single stage reactor. Although the two-stage approach appears attractive for microalgae-based biofuel production based on successful studies, its feasibility for large-scale outdoor commercial operations is questionable due to high energy demands and costs deriving from the necessity of managing two reactors instead of one [42].

6. Microalgae harvesting

Cultivated microalgae needs to be separated from the cultivation broth to be further used and the harvesting is one of the costliest stages in entire microalgae-based process, making its optimization essential for improving process sustainability. The elevated costs primarily due to the high-water content of the cell broth after cultivation [127]. Insufficient water recovery becomes a critical issue, given its high consumption rates. Hence, it is crucial to identify appropriate techniques for harvesting microalgae and recycling the separated medium to enhance process sustainability. In PBRs, the bulk culture medium typically contains a significant water content ranging from 99.5 % to 99.9 %. Therefore, biomass harvesting is mainly achieved through a dewatering/concentrating procedure. This operating phase usually involves a two-step process. The first step, known as bulk harvesting, concentrates the biomass to 2 %–7 % dry weight. The second step, called thickening, further concentrates the algal slurry to about 15–25 %, making it more manageable for subsequent downstream operations [128]. A summary of the main techniques for microalgae harvesting is provided in Table 4.

The efficiency of harvesting is closely tied to the size of particles or particle aggregates (flocs) that need to be separated from the liquid phase. Filtration systems can only separate flocs that are larger than the pores of membranes or cakes used in the process. Conversely, in gravity settling or centrifuges, the recovery yields depend on how quickly flocs move in response to the mechanical force field acting on the particles, which is determined by the fluid dynamics of the system. Here, the settling rate follows the well-known Stokes law and is directly proportional to the size of particles or flocs. Therefore, larger flocs settle faster, resulting in higher recovery yields. For this reason, a flocculation step is often introduced before harvesting, aiming to promote microalgae aggregation and enlarge floc sizes. Understanding the size distribution of cells/flocs in the microalgal suspension is crucial for simulating, designing, or controlling the aforementioned processes. To address this need, some literature works have focused on developing Population Balance Equations (PBE) to assess the evolution of microalgae (or microalgae aggregates) size evolution during the flocculation and harvesting stages of the operation.

7. Downstream processes

While this review focuses on the cultivation step for BJFs production from microalgae it is important to mention that several operations should performed downstream to obtain a BJF compatible with the current aircrafts. In this view, once microalgal biomass is harvested, it can be processed in two ways: drying or using it directly as wet biomass, depending on the subsequent process chosen to produce BJFs. However, it is important to note that drying can be a costly step due to the high energy consumption involved. Therefore, it is generally more favourable to opt for downstream processes that can directly utilize wet biomass as input, thus avoiding the need for drying. Fig. 5 shows the main downstream processes adopted to produce BJF from microalgae.

More innovative options for producing BJF from microalgal feedstocks include Fischer-Tropsch synthesis after microalgal biomass gasification [5], alcohol production through fermentation of carbohydrate microalgal fraction [5], and terpene production [129]. Despite their potential, the use of these techniques for producing BJF from microalgae is still at an early stage of development. To meet the minimum requirements for BJF production, the bio-oils obtained from the three cited fractions (extraction, hydrothermal liquefaction, and pyrolysis) need to undergo upgrading processes. These processes aim to reduce unsaturation in C chains, lower the oxygen content, crack C chains, and isomerize hydrocarbons [130].

Currently, two processes for producing BJF from microalgae have been certified according to ASTMD7566 [131]:

- HEFA (hydrotreated esters and fatty acids) from oily biomass, such as algae, jatropha, camelina. Since 2011 HEFA can be used as blending component up to 50 vol% in crude-oil kerosene mainly due to its poor cold flow properties, and absence of aromatics.
- HHC-SPK or HC-HEFA-SPK (Hydroprocessed Hydrocarbons, Esters and Fatty Acids Synthetic Paraffinic Kerosene) from hydrocarbons of biological origin, fatty acid esters, free fatty acids, or a species of *Botryococcus braunii* algae. Since 2020, it can be used as a blending component of up to 10 %

The detailed treatment on downstream processes goes beyond the scope of this work which is focused on the cultivation steps, so it won't be discussed further.

8. Techno economic and LCA aspects

No existing study in the literature has estimated the total costs of producing BJF form microalgae, covering cultivation to oil upgrade. Therefore, this analysis will focus on costs, net energy ratio (NER), and life cycle assessment (LCA) for generic biofuel production from microalgae. It is noteworthy that significant costs are incurred in microalgae cultivation and harvesting, underscoring the need for techno-economic optimization to ensure the whole process sustainability.

8.1. Biofuel production cost

The application of microalgae-based biofuels on an industrial scale necessitates achieving a competitive production cost to match the selling price of conventional fuels. Conventional petroleum-based aviation fuel is priced at around 800 € ton^{-1} [132], approximately 0.02 € MJ^{-1}.

For the possibility of producing BJF from microalgae, pilot-scale tests ranging from 0.5 to 40 m^3 allowed to estimate microalgal biomass production costs between 3 – 6 € kg^{-1} [133,134]. This cost range was estimated considering different process configurations (flat panels,

Table 4
Comparison of main harvesting methods for algae, adapted from Concas et al. [26].

Method	Post harvest concentration	Recovery yields	Major benefits	Major limitations
Centrifugation	12–22 wt%	> 90 %	Reliable, high solids conc.	Energy-intensive, high cost
Tangential filtration	5–27 wt%	70–90 %	Reliable, high solids conc.	Membrane fouling, high cost
Gravity sedimentation	0.5–3 wt%	10–90 %	Low cost	Slow, unreliable
Dissolved air flotation	3–6 wt%	50–90 %	Proven at large scale	Flocculants usually required

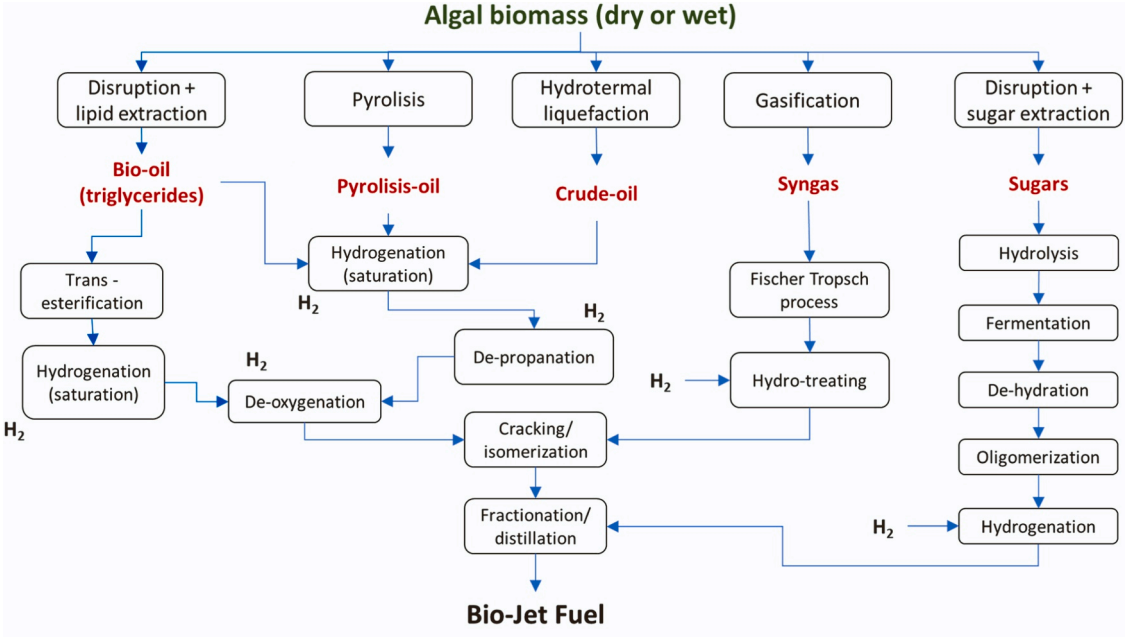

Fig. 5. Main downstream processes for the production of BJFs from microalgal biomass.

tubular PBRs, open ponds), in different geographical regions, testing different microalgal strains. Some authors have speculated the potential to reduce costs approximately 0.5 € kg^{-1} in the future, contingent upon further technical advancements [133]. Heterotrophic fermentation of organic substrates offers a potentially viable production method, with estimated costs ranging from 1.1 to 4.0 € kg^{-1} [135,136]. Despite these estimates, the actual production volumes for economic assessments remain relatively small compared to active industrial production plants. For instance, European microalgae production in 2021 was approximately 300 tons yr^{-1}, primarily used in high-value products like food and feed supplements [137]. For applications to low-cost commodities like biofuels, the market demand would be remarkably higher. For renewable jet fuels, a request jump from 10^9 MJ in 2021–10^{11} MJ in 2030, corresponding to 3.8 – 6.1 Mt yr^{-1} is expected [138].

Regarding the economics of microalgal cultivation systems, open ponds are cheaper for biomass synthesis but require more energy for harvesting compared to PBRs [133]. Microalgal biomass has an energy content of around 20–25 MJ kg^{-1} [139], half than conventional JFs (43 MJ kg^{-1}) [5,140], necessitating conversion into more energy-dense products. Wet microalgal biomass production costs of 3–6 € kg^{-1} dry biomass equivalent [133,134] corresponds to a cost per unit energy of 0.12 – 0.30 € MJ^{-1}, which is 4–10 folds higher than conventional JFs.

Biorefinery operations to convert microalgal biomass to biofuels add further costs, with estimates ranging from 0.4 to 1.8 € kg^{-1} for biodiesel production from microalgal lipids [133]. Additional processes, such as hydroprocessing, are required to obtain fuels meeting JF specifications [5]. Thermochemical conversions like hydrothermal liquefaction (HTL) offer promising methods to convert microalgal biomass into bio-oil. HTL produces a bio-oil with a higher heating value (HHV) of 29–35 MJ kg^{-1} [141], which requires further refining to meet JF standards [142]. The estimated cost of HTL is around 0.93€ per Gasoline Gallon Equivalent (GGE) [142] i.e. about 0.008 € MJ^{-1}, which is less relevant compared to the production cost of microalgae biomass. Recent studies have employed detailed models to predict biofuel selling prices under variable climatic and economic conditions, with minimum estimates around 1.85 € L^{-1} [143]. These efforts underscore the ongoing challenge of making microalgae-based biofuels economically viable on industrial scale.

8.2. Energy efficiency

Assessing the energy efficiency of biofuel production is crucial, often done using the Net Energy Ratio (NER), which compares the output energy in biofuel to the input energy supplied to the process.

$$NER = \frac{Energy\ output}{\sum Energy\ input}$$

For a process to be energetically favourable and technically feasible, NER should be > 1, indicating an energy gain. As a reference, diesel from fossil sources typically has a NER around 5 [144]. Microalgae cultivation processes require energy for various tasks like culture mixing, cleaning, aeration, cooling, heating, harvesting. However, the energy consumed should be lower than the energy stored inside microalgae biomass via photosynthesis. Studies in the literature present contradictory data on NER, with some reporting values of NER > 1, comparable to diesel,

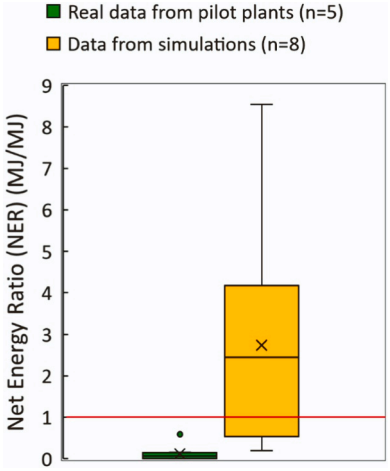

Fig. 6. Net Energy Ratio as determined in different previous papers published between 2012 and 2022. NER is calculated as output energy/input energy. With n the number of source papers is indicated. For some papers more values have been taken, since different configurations were assessed. In total 11 values for simulation and 8 values for real pilot plants were included in the figure.

while others report values much lower. An overview of this variability can be viewed in Fig. 6.

Studies available in the literature can be divided into these calculating NER from real data obtained from pilot plants and those using hypothetical plant designs with estimated energy consumptions and biomass productivities. Data from simulations often yield NER > 1 (average: 2.7, median: 2.45) [145–151], while data from real plants typically yield NER < 1 (average: 0.16, median: 0.07) [152–156]. This discrepancy is due to differences in approach and assumptions, with simulations sometimes underestimating energy input or overestimating biomass productivity. Among authors who used simulations, some authors (only a minor part) estimated biomass productivity by using mechanistic models to relate growth rate to the environmental variable conditions and technical properties of the specific plant, while other authors (the large majority) just assumed arbitrarily a certain biomass productivity. In many simulations, biomass productivity (g m^{-2} d^{-1}) was fixed as a constant value based on previous studies or tests at laboratory scale, which are not well representative of industrial conditions [147, 149,150,157–159]. Real plants data from different geographical locations (Spain, Italy, The Netherlands) indicate a wide range of cumulative energy demand between 38 MJ kg^{-1} and 70,164 MJ kg^{-1} and a high influence of environmental conditions (solar radiation and temperature) on determining the NER [153,154,156].

Energy consumption per unit of produced biomass can vary significantly depending on environmental conditions like solar radiation and temperature [153,160]. Aeration is also a major energy factor to be considered, with a compromise needed between energy consumption and growth rate [151] The best NER found from real plant data was 0.59, obtained with *Tetraselmis suecica* in Green Wall Panel (GWP-II), in Italy [151]. In this case, the input energy came for 59 % from operative activities (of which 68 % from blowers), 30 % embodied in used materials (mainly bioreactor construction) and 11 % from fertilizers (N and P) synthesis [151].

Improvements cultivation technologies, such as selecting better geographical sites with favourable weather conditions, using more resistant and productive strains, process integration, and improvements in materials and reactor design, could potentially help achieve NER > 1 in the future. However, uncertainties remain, and further research is needed to improve the energy efficiency of microalgae-based biofuel production

8.3. LCA aspects

LCA analysis provides a holistic view of the environmental impact of a process, considering indicators like energy consumption, GHG emissions, water footprint, land use, and resource depletion. In the context of microalgae-based biofuels, LCA studies have focused primarily on energy cost and GHG emissions, with less attention to water footprint, land use, and resource depletion [161]. As baseline, the reference GHG emission of conventional fossil fuels is 83.8 g CO_2 eq. MJ^{-1} for EU Directive and 93.3 g CO_2 eq. MJ^{-1} for US standards [161,162] For water footprint, the reference value for petroleum-based diesel is 0.1 m^3 MJ^{-1} [163].

Meta-analysis of LCA studies have shown wide ranges of values for energy costs and GHG emissions, indicating significant variability in environmental impacts [163]. A 2017 meta-analysis evaluated and compared LCA results from 54 studies (2009–2016), after data harmonization, to minimize study-to-study differences [163]. A very wide range of values between −4.3 kg CO_2 eq. MJ^{-1} and +9.2 kg CO_2 eq. MJ^{-1} was found. In this range, the values below the fossil fuel baseline were 30–40 % of total. A recent report on algae and climate published in 2023 by the European Commission reported a total carbon footprint (direct and indirect CO_2 emissions) from 21 to 1087 kg CO_2 per kg of dry microalgae biomass. Water consumption for microalgae fuels have also varied widely, between – 0.05–1.02 m^3 MJ^{-1}, with 93 % of data below the fossil fuel baseline [163]. However, many LCA studies suffer from limitations, such as arbitrary parameter settings and reliance on non-representative reference cases. This approach can significantly affect the reliability of the results and the accuracy of environmental impact assessments. Land use can also have relevant environmental impacts, particularly through direct land use change resulting from microalgae facility construction [164]. This can significantly increase GHG emissions and other environmental pressures associated with land use change [164]. Resource depletion is another important consideration, particularly for nutrients like N and P used in microalgae cultivation [138]. Considering an estimated request for renewable JF in European Union of 3.8 – 6.1 Mt yr^{-1} [138], and a fuel to biomass yield of 0.3, about 1–1.6 Mt yr^{-1} of N and 0.1–0.2 Mt yr^{-1} of P would be required. P is classified as a critical raw material (CRM) in Europe, since its large use in agriculture and industry and its scarcity in nature. The European annual use of N and P fertilizers have been 10 Mt yr^{-1} and 1.2 Mt yr^{-1}, respectively, in 2020. The production of JF could therefore affect about 10–20 % of the fertilizer production capacity. Since JF does not contain N and P, recycled nutrients from microalgae biomass processing, such as during HTL, can contribute to a circular economy strategy and reduce reliance on fertilizers [165]. Overall, while LCA provides valuable insights into the environmental impacts of microalgae-based biofuels, there is a need for further research to address methodological limitations and improve the accuracy and reliability of assessments.

9. Conclusions

The use of microalgae for generating BJF stands as a promising opportunity to decarbonize the aviation sector. Specific microalgal strains (i.e. *Isochrysis galbana*, *Pavlova salina*, *Chaetoceros* sp., and others) can produce compounds useful as BJF precursors, but their biomass productivity can be limited. Strategies to promote lipid accumulation in these strains often rely on the adoption of stress conditions, such as light stress, extreme temperatures, and nutrient starvation, which can enhance lipid accumulation up to 40 % of the biomass dry weight. Nonetheless, prolonged stress can lead to the breakdown of the photosynthetic apparatus, chlorophyll degradation, inhibition of cell division, and overall reduction of productivity. Optimizing cultivation conditions is crucial, with open ponds being more frequently used to cultivate microalgae for fuel production, even though less productive compared to closed systems (i.e. PBRs)., Closed raceway ponds can provide the advantages of PBRs, while keeping the low costs of open systems. In this regard, the identification of engineering solutions that can integrate the process optimization with the operating costs reduction is of paramount importance to make microalgal-based BJF competitive with fossil fuels. Using WW and off-gases to support microalgal metabolism aligns with the principles of the circular economy. Microalgae can uptake, indeed, the carbon and/or the N for their growth from either gaseous or liquid emissions, whose content of these nutrients need to be reduced before their sustainable release into the environment. Future studies should unveil the possible adverse effects of contaminants in WW and high concentrations of toxic gaseous components on microalgal growth and lipid productivity.

CRediT authorship contribution statement

Adriana Ciurli: Writing – original draft, Investigation, Formal analysis, Data curation. **Alessandro Concas:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Carolina Chiellini:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Fabrizio Di Caprio:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Francesca Pagnanelli:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ali Parsaeimehr:** Writing – original draft, Investigation, Formal analysis, Data curation. **Ilze Malina:** Writing – original draft,

Investigation, Formal analysis, Data curation. **Kristaps Malins:** Writing – original draft, Investigation, Formal analysis, Data curation. **Massimiliano Fabbricino:** Writing – original draft, Investigation, Formal analysis, Data curation. **Giacomo Cao:** Writing – review & editing, Formal analysis, Data curation. **Alessandra Cesaro:** Writing – original draft, Investigation, Formal analysis, Data curation. **Giovanni Antonio Lutzu:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Grazia Policastro:** Writing – original draft, Formal analysis, Data curation. **Luca Usai:** Writing – original draft, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

All the authors would like to extend a very special thanks to the great soul of Carolina Chiellini for her intelligence, diligence, strength and tenacity. Carolina gave a fundamental and exceptional contribution during the preparation of this paper, even though unfortunately she hasn't had the time to see this finally published. This review is therefore dedicated to her memory with all our love.

A special acknowledgement is also address towards Dr. Teodoro Francia for his kind contribution in providing a hand-made china ink for the graphical abstract.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2024.113886.

References

[1] R.S. Capaz, E. Guida, J.E.A. Seabra, P. Osseweijer, J.A. Posada, Mitigating carbon emissions through sustainable aviation fuels: costs and potential, Biofuels, Bioprod. Bioref. (2021), https://doi.org/10.1002/bbb.2168.

[2] K. Atsonios, M.A. Kougioumtzis, K. D. Panopoulos, E. Kakaras, Alternative thermochemical routes for aviation biofuels via alcohols synthesis: process modeling, techno-economic assessment and comparison, Appl. Energy (2015), https://doi.org/10.1016/j.apenergy.2014.10.056.

[3] A. Atmanli, Experimental comparison of biodiesel production performance of two different microalgae, Fuel (2020), https://doi.org/10.1016/j.fuel.2020.118311.

[4] M. Branco-Vieira, S.S. Martin, C. Agurto, M.A. Santos, M.A.V. Freitas, N. S. Caetano, Analyzing *Phaeodactylum tricornutum* lipid profile for biodiesel production, : Energy Procedia (2017), https://doi.org/10.1016/j.egypro.2017.10.251.

[5] J.H.K. Lim, Y.Y. Gan, H.C. Ong, B.F. Lau, W.H. Chen, C.T. Chong, T.C. Ling, J. J. Klemeš, Utilization of microalgae for bio-jet fuel production in the aviation sector: challenges and perspective, Renew. Sustain. Energy Rev. 149 (2021) 111396.

[6] Z. Shi, B. Zhao, S. Tang, X. Yang, Hydrotreating lipids for aviation biofuels derived from extraction of wet and dry algae, J. Clean. Prod. (2018), https://doi.org/10.1016/j.jclepro.2018.08.351.

[7] M. Mofijur, S.M. Ashrafur Rahman, L.N. Nguyen, T.M.I. Mahlia, L.D. Nghiem, Selection of microalgae strains for sustainable production of aviation biofuel, Bioresour. Technol. 345 (2022), https://doi.org/10.1016/j.biortech.2021.126408.

[8] V.T. Duong, F. Ahmed, S.R. Thomas-Hall, S. Quigley, E. Nowak, P.M. Schenk, High protein- and high lipid-producing microalgae from northern Australia as potential feedstock for animal feed and biodiesel, Front. Bioeng. Biotechnol. (2015), https://doi.org/10.3389/fbioe.2015.00053.

[9] A. Gómez-De la Cruz, A.G. Romero-Izquierdo, C. Gutiérrez-Antonio, F.I. Gómez-Castro, S. Hernández, Modelling of the hydrotreating process to produce renewable aviation fuel from micro-algae oil, Comput. Aided Chem. Eng. (2017), https://doi.org/10.1016/B978-0-444-63965-3.50111-2.

[10] J.K. Bwapwa, A. Anandraj, C. Trois, Possibilities for conversion of microalgae oil into aviation fuel: a review, Renew. Sustain. Energy Rev. 80 (2017) 1345–1354, https://doi.org/10.1016/j.rser.2017.05.224.

[11] D.K.Y. Lim, S. Garg, M. Timmins, E.S.B. Zhang, S.R. Thomas-Hall, H. Schuhmann, Y. Li, P.M. Schenk, Isolation and evaluation of oil-producing microalgae from subtropical coastal and Brackish waters, PLoS One (2012), https://doi.org/10.1371/journal.pone.0040751.

[12] L. Tejeda-Benítez, D. Henao-Argumedo, M. Alvear-Alayón, C.R. Castillo-Saldarriaga, Characterization and lipid profile of oil from microalgae Caracterização e perfil lipídico de azeites de microalgas, Rev. Fac. Ing. (Fac. Ing.) (2015).

[13] C. Gutiérrez-Antonio, A. Gómez-De la Cruz, A.G. Romero-Izquierdo, F.I. Gómez-Castro, S. Hernández, Modeling, simulation and intensification of hydroprocessing of micro-algae oil to produce renewable aviation fuel, Clean. Technol. Environ. Policy (2018), https://doi.org/10.1007/s10098-018-1561-z.

[14] T.H. Kim, K. Lee, B.R. Oh, M.E. Lee, M. Seo, S. Li, J.K. Kim, M. Choi, Y.K. Chang, A novel process for the coproduction of biojet fuel and high-value polyunsaturated fatty acid esters from heterotrophic microalgae *Schizochytrium* sp. ABC101, Renew. Energy (2021), https://doi.org/10.1016/j.renene.2020.09.116.

[15] Z. Habibzadeh, V. Babaeipour, S.G. Pakdehi, P. Sazandechi, Optimization of biodiesel production process from household waste oil, rapeseed, and microalgae oils as a suitable alternative for jet-fuel, (2022).

[16] Z. Shokravi, H. Shokravi, O.H. Chyuan, W.J. Lau, S.S.R. Koloor, M. Petr\uu, A. F. Ismail, Improving 'lipid productivity'in microalgae by bilateral enhancement of biomass and lipid contents: a review, Sustainability 12 (2020) 9083.

[17] N. Arora, P.T. Pienkos, V. Pruthi, K.M. Poluri, M.T. Guarnieri, Leveraging algal omics to reveal potential targets for augmenting {TAG} accumulation, Biotechnol. Adv. 36 (2018) 1274–1292.

[18] A. Usai, C. Theodoropoulos, F. Di Caprio, P. Altimari, G. Cao, A. Concas, Structured population balances to support microalgae-based processes: review of the state-of-art and perspectives analysis, Comput. Struct. Biotechnol. J. (2023).

[19] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sustain. Energy Rev. 14 (2010) 217–232, https://doi.org/10.1016/J.RSER.2009.07.020.

[20] Q. Kong, L. Li, B. Martinez, P. Chen, R. Ruan, Culture of microalgae Chlamydomonas reinhardtii in wastewater for biomass feedstock production, Appl. Biochem. Biotechnol. 160 (2010) 9–18.

[21] S.P. Slocombe, Q. Zhang, M. Ross, A. Anderson, N.J. Thomas, Á. Lapresa, C. Rad-Menéndez, C.N. Campbell, K.D. Black, M.S. Stanley, J.G. Day, Unlocking nature's treasure-chest: screening for oleaginous algae, Sci. Rep. 5 (2015) 1–17.

[22] Y. Feng, C. Li, D. Zhang, Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium, Bioresour. Technol. 102 (2011) 101–105.

[23] A.F. Talebi, S.K. Mohtashami, M. Tabatabaei, M. Tohidfar, A. Bagheri, M. Zeinalabedini, H. Hadavand Mirzaei, M. Mirzajanzadeh, S. Malekzadeh Shafaroudi, S. Bakhtiari, Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production, Algal Res 2 (2013) 258–267, https://doi.org/10.1016/J.ALGAL.2013.04.003.

[24] L. Rodolfi, G. Chini Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, M. R. Tredici, Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, Biotechnol. Bioeng. 102 (2009) 100–112.

[25] C.-Y. Chen, K.-L. Yeh, R. Aisyah, D.-J. Lee, J.-S. Chang, Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review, Bioresour. Technol. 102 (2011) 71–81, https://doi.org/10.1016/j.biortech.2010.06.159.

[26] A. Concas, M. Pisu, G. Cao, Engineering Aspects Related to the Use of Microalgae for Biofuel Production and CO2 Capture from Flue Gases Springer, Curr. Environ. Issues Challenges (2014) 73–111.

[27] S.-Y. Chiu, M.-T. Tsai, C.-Y. Kao, S.-C. Ong, C.-S. Lin, The air-lift photobioreactors with flow patterning for high-density cultures of microalgae and carbon dioxide removal, Eng. Life Sci. 9 (2009) 254–260.

[28] L.M. González-González, D.F. Correa, S. Ryan, P.D. Jensen, S. Pratt, P.M. Schenk, Integrated biodiesel and biogas production from microalgae: towards a sustainable closed loop through nutrient recycling, Renew. Sustain. Energy Rev. 82 (2018) 1137–1148.

[29] L. Gouveia, A.C. Oliveira, R. Congestri, L. Bruno, A.T. Soares, R.S. Menezes, N.R. A. Filho, I. TzovenisBiodiesel from microalgae Elsevier , Microalgae-Based Biofuels Bioprod , 2017, , 235–258, 10.1016/B978-0-08-101023-5.00010-8.

[30] J.P. Maity, J. Bundschuh, C.-Y. Chen, P. Bhattacharya, Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – a mini review, Energy 78 (2014) 104–113, https://doi.org/10.1016/J.ENERGY.2014.04.003.

[31] S. Soru, V. Malavasi, P. Caboni, A. Concas, G. Cao, Behavior of the extremophile green alga *Coccomyxa melkonianii* SCCA 048 in terms of lipids production and morphology at different pH values, Extremophiles (1) (2018) 79–89, https://doi.org/10.1007/s00792-018-1062-3.

[32] A.K. Minhas, P. Hodgson, C.J. Barrow, A. Adholeya, A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids, Front. Microbiol. 7 (2016) 1–19.

[33] L. Zhu, Y.K. Nugroho, S.R. Shakeel, Z. Li, B. Martinkauppi, E. Hiltunen, Using microalgae to produce liquid transportation biodiesel: what is next? Renew. Sustain. Energy Rev. 78 (2017) 391–400.

[34] R. Radakovits, R.E. Jinkerson, S.I. Fuerstenberg, H. Tae, R.E. Settlage, J.L. Boore, M.C. Posewitz, Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropis gaditana*, Nat. Commun. 3 (2012) 686.

[35] A.L. Gonçalves, J.C.M. Pires, M. Simões, Lipid production of *Chlorella vulgaris* and Pseudokirchneriella subcapitata, Int. J. Energy Environ. Eng. 4 (2013) 14.

[36] S.K. Mandotra, P. Kumar, M.R. Suseela, S. Nayaka, P.W. Ramteke, Evaluation of fatty acid profile and biodiesel properties of microalga *Scenedesmus abundans* under the influence of phosphorus, pH and light intensities, Bioresour. Technol. 201 (2016) 222–229.

[37] I. Saadaoui, M. Cherif, R. Rasheed, T. Bounnit, H. Al Jabri, S. Sayadi, R. Ben Hamadou, S.R. Manning, *Mychonastes homosphaera* (Chlorophyceae): a promising feedstock for high quality feed production in the arid environment, Algal Res (2020), https://doi.org/10.1016/j.algal.2020.102021.

[38] T. Takeshita, S. Ota, T. Yamazaki, A. Hirata, V. Zachleder, S. Kawano, Starch and lipid accumulation in eight strains of six *Chlorella* species under comparatively high light intensity and aeration culture conditions, Bioresour. Technol. 158 (2014) 127–134.

[39] D. Pal, I. Khozin-Goldberg, Z. Cohen, S. Boussiba, The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp, Appl. Microbiol. Biotechnol. 90 (2011) 1429–1441.

[40] S.V. Khotimchenko, I.M. Yakovleva, Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance, Phytochemistry 66 (2005) 73–79.

[41] S. Kumari, S. Satapathy, M. Datta, S. Kumar, Adaptation of Microalgae to Temperature and Light Stress, Adv. Sci. Technol. Innov. (2022), https://doi.org/10.1007/978-3-030-95365-2_8.

[42] S.-H. Ho, X. Ye, T. Hasunuma, J.-S. Chang, A. Kondo, Perspectives on engineering strategies for improving biofuel production from microalgae–a critical review, Biotechnol. Adv. 32 (2014) 1448–1459.

[43] A. Udayan, A.K. Pandey, R. Sirohi, N. Sreekumar, B.I. Sang, S.J. Sim, S.H. Kim, A. Pandey, Production of microalgae with high lipid content and their potential as sources of nutraceuticals, Phytochem. Rev. 2021 (2022) 1–28, https://doi.org/10.1007/S11101-021-09784-Y.

[44] L.D. Zhu, Z.H. Li, E. Hiltunen, Strategies for lipid production improvement in microalgae as a biodiesel feedstock, Biomed. Res. Int. 2016 (2016) 8792548.

[45] L. Xin, H. Hong-ying, Z. Yu-ping, Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. under different cultivation temperature, Bioresour. Technol. 102 (2011) 3098–3102.

[46] J. Cao, H. Yuan, B. Li, J. Yang, Significance evaluation of the effects of environmental factors on the lipid accumulation of *Chlorella minutissima* UTEX 2341 under low-nutrition heterotrophic condition, Bioresour. Technol. 152 (2014) 177–184.

[47] A. Converti, A.A. Casazza, E.Y. Ortiz, P. Perego, M. Del Borghi, Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production, Chem. Eng. Process. Process. Intensif. 48 (2009) 1146–1151.

[48] M. Vitova, K. Bisova, S. Kawano, V. Zachleder, Accumulation of energy reserves in algae: from cell cycles to biotechnological applications, Biotechnol. Adv. 33 (2015) 1204–1218.

[49] Y. Luo, Y. Ding, X. Jiang, G. Zeng, R. Peng, Q. Han, M. Jiang, Effects of low temperature and highlight stress on lipid accumulation and cell structure of *Tropidoneis maxima*, J. Basic Microbiol. (2023).

[50] L. Wei, X. Huang, Z. Huang, Temperature effects on lipid properties of microalgae *Tetraselmis subcordiformis* and *Nannochloropsis oculata* as biofuel resources, Chin. J. Ocean. Limnol. 33 (2015) 99–106.

[51] H. Chen, Q. Wang, Regulatory mechanisms of lipid biosynthesis in microalgae, Biol. Rev. (2021), https://doi.org/10.1111/brv.12759.

[52] G. Mujtaba, W. Choi, C.-G. Lee, K. Lee, Lipid production by *Chlorella vulgaris* after a shift from nutrient-rich to nitrogen starvation conditions, Bioresour. Technol. 123 (2012) 279–283.

[53] Z. Zhu, J. Sun, Y. Fa, X. Liu, P. Lindblad, Enhancing microalgal lipid accumulation for biofuel production, Front. Microbiol. (2022), https://doi.org/10.3389/fmicb.2022.1024441.

[54] S. Mandal, N. Mallick, Microalga *Scenedesmus obliquus* as a potential source for biodiesel production, Appl. Microbiol. Biotechnol. 84 (2009) 281–291.

[55] A. Sato, R. Matsumura, N. Hoshino, M. Tsuzuki, N. Sato, Responsibility of regulatory gene expression and repressed protein synthesis for triacylglycerol accumulation on sulfur-starvation in *Chlamydomonas reinhardtii*, Front. Plant Sci. 5 (2014) 444.

[56] S. Soru, V. Malavasi, A. Concas, P. Caboni, G. Cao, A novel investigation of the growth and lipid production of the extremophile microalga *Coccomyxa melkonianii* SCCA 048 under the effect of different cultivation conditions: experiments and modeling, Chem. Eng. J. 377 (2019) 120589, https://doi.org/10.1016/j.cej.2018.12.049.

[57] A. Concas, V. Malavasi, C. Costelli, P. Fadda, M. Pisu, G. Cao, Autotrophic growth and lipid production of *Chlorella sorokiniana* in lab batch and BIOCOIL photobioreactors: experiments and modeling, Bioresour. Technol. 211 (2016) 327–338, https://doi.org/10.1016/j.biortech.2016.03.089.

[58] F. Mairet, O. Bernard, P. Masci, T. Lacour, A. Sciandra, Modelling neutral lipid production by the microalga *Isochrysis aff. galbana* under nitrogen limitation, Bioresour. Technol. 102 (2011) 142–149.

[59] S. Soru, V. Malavasi, A. Concas, P. Caboni, G. Cao, Modeling and experimental investigation of the effect of nitrogen starvation and pH variation on the cultivation of the extremophile microalga *Coccomyxa melkonianii* SCCA048, Chem. Eng. Trans. 74 (2019), https://doi.org/10.3303/CET1974033.

[60] P. Darvehei, P.A. Bahri, N.R. Moheimani, Model development for the growth of microalgae: a review, Pergamon (2018), https://doi.org/10.1016/j.rser.2018.08.027.

[61] A. Concas, A. Steriti, M. Pisu, G. Cao, Experimental and theoretical investigation of the effects of iron on growth and lipid synthesis of microalgae in view of their use to produce biofuels, J. Environ. Chem. Eng. 9 (2021) 105349, https://doi.org/10.1016/j.jece.2021.105349.

[62] M. Bekirogullari, G.M. Figueroa-Torres, J.K. Pittman, C. Theodoropoulos, Models of microalgal cultivation for added-value products - a review, Biotechnol. Adv. 44 (2020) 107609, https://doi.org/10.1016/j.biotechadv.2020.107609.

[63] E. Lee, M. Jalalizadeh, Q. Zhang, Growth kinetic models for microalgae cultivation: a review, Algal Res 12 (2015) 497–512, https://doi.org/10.1016/J.ALGAL.2015.10.004.

[64] K. Meenatchisundaram, S.C. Gowd, J. Lee, S. Barathi, K. Rajendran, Data-driven model development for prediction and optimization of biomass yield of microalgae-based wastewater treatment, Sustain. Energy Technol. Assess. (2024), https://doi.org/10.1016/j.seta.2024.103670.

[65] T. Syed, F. Krujatz, Y. Ihadjadene, G. Mühlstädt, H. Hamedi, J. Mädler, L. Urbas, A review on machine learning approaches for microalgae cultivation systems, Comput. Biol. Med. (2024), https://doi.org/10.1016/j.compbiomed.2024.108248.

[66] P.J. le, B. Williams, L.M.L. Laurens, Microalgae as biodiesel and biomass feedstocks: Review and analysis of the biochemistry, energetics and economics, Energy Environ. Sci. 3 (2010) 554, https://doi.org/10.1039/b924978h.

[67] G. Breuer, P.P. Lamers, D.E. Martens, R.B. Draaisma, R.H. Wijffels, The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains, Bioresour. Technol. 124 (2012) 217–226, https://doi.org/10.1016/j.biortech.2012.08.003.

[68] A.T. Hoang, R. Sirohi, A. Pandey, S. Nižetić, S.S. Lam, W.-H. Chen, R. Luque, S. Thomas, M. Ar\ic\i, V.V. Pham, Biofuel production from microalgae: challenges and chances, Phytochem. Rev. (2022) 1–38.

[69] P.W. Behrens, Photobioreactors and fermentors: the light and dark sides of growing algae, Algal Cult. Tech. (2005).

[70] E. Suali, R. Sarbatly, Conversion of microalgae to biofuel, Renew. Sustain. Energy Rev. 16 (2012) 4316–4342.

[71] A.K. Sharma, P.K. Sahoo, S. Singhal, A. Patel, Impact of various media and organic carbon sources on biofuel production potential from *Chlorella* spp, 3 Biotech (2016), https://doi.org/10.1007/s13205-016-0434-6.

[72] F. Di Caprio, P. Altimari, G. Iaquaniello, L. Toro, F. Pagnanelli, Heterotrophic cultivation of *T. obliquus* under non-axenic conditions by uncoupled supply of nitrogen and glucose, Biochem. Eng. J. 145 (2019) 127–136, https://doi.org/10.1016/j.bej.2019.02.020.

[73] O. Perez-Garcia, F.M.E. Escalante, L.E. de-Bashan, Y. Bashan, Heterotrophic cultures of microalgae: metabolism and potential products, Water Res (2011), https://doi.org/10.1016/j.watres.2010.08.037.

[74] X.M. Shi, Y. Jiang, F. Chen, High-yield production of lutein by the green microalga *Chlorella protothecoides* in heterotrophic fed-batch culture, Biotechnol. Prog. (2002), https://doi.org/10.1021/bp0101987.

[75] F.J. Choix, L.E. de-Bashan, Y. Bashan, Enhanced accumulation of starch and total carbohydrates in alginate-immobilized *Chlorella* spp. induced by *Azospirillum brasilense*: II. Heterotrophic conditions, Enzym. Microb. Technol. (2012), https://doi.org/10.1016/j.enzmictec.2012.07.012.

[76] Y. Zheng, Z. Chi, B. Lucker, S. Chen, Two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production, Bioresour. Technol. (2012), https://doi.org/10.1016/j.biortech.2011.09.122.

[77] Y.-K. Lee, H. Shen, Basic Culturing Techniques, Handb. Micro Cult. (2007) 40, https://doi.org/10.1002/9780470995280.ch3.

[78] W. Xiong, X. Li, J. Xiang, Q. Wu, High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production, Appl. Microbiol. Biotechnol. 78 (2008) 29–36.

[79] X. Miao, Q. Wu, High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*, J. Biotechnol. (2004), https://doi.org/10.1016/j.jbiotec.2004.01.013.

[80] H. Xu, X. Miao, Q. Wu, High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters, J. Biotechnol. 126 (2006) 499–507.

[81] M.E. De Swaaf, J.T. Pronk, L. Sijtsma, Fed-batch cultivation of the docosahexaenoic-acid-producing marine alga *Crypthecodinium cohnii* on ethanol, Appl. Microbiol. Biotechnol. (2003), https://doi.org/10.1007/s00253-002-1118-1.

[82] O.S. Graverholt, N.T. Eriksen, Heterotrophic high-cell-density fed-batch and continuous-flow cultures of *Galdieria sulphuraria* and production of phycocyanin, Appl. Microbiol. Biotechnol. (2007), https://doi.org/10.1007/s00253-007-1150-2.

[83] A.M. Aguirre, A. Bassi, P. Saxena, Engineering challenges in biodiesel production from microalgae, Crit. Rev. Biotechnol. (2013), https://doi.org/10.3109/07388551.2012.695333.

[84] M. Wan, P. Liu, J. Xia, J.N. Rosenberg, G.A. Oyler, M.J. Betenbaugh, Z. Nie, G. Qiu, The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in *Chlorella sorokiniana*, Appl. Microbiol. Biotechnol. (2011), https://doi.org/10.1007/s00253-011-3399-8.

[85] M.R. Andrade, J.A.V. Costa, Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate, Aquaculture (2007), https://doi.org/10.1016/j.aquaculture.2006.11.021.

[86] C. Yang, Q. Hua, K. Shimizu, Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions, Biochem. Eng. J. (2000), https://doi.org/10.1016/S1369-703X(00)00080-2.

[87] Y.K. Lee, S.Y. Ding, C.H. Hoe, C.S. Low, Mixotrophic growth of *Chlorella sorokiniana* in outdoor enclosed photobioreactor, J. Appl. Phycol. (1996), https://doi.org/10.1007/BF02186320.

[88] W.H. Leong, J.W. Lim, M.K. Lam, S.M. Lam, J.C. Sin, A. Samson, Novel sequential flow baffled microalgal-bacterial photobioreactor for enhancing nitrogen assimilation into microalgal biomass whilst bioremediating nutrient-rich wastewater simultaneously, J. Hazard. Mater. (2021), https://doi.org/10.1016/j.jhazmat.2020.124455.

[89] A. Concas, G.A. Lutzu, N. Turgut Dunford, Experiments and modeling of *Komvophoron* sp. Growth in hydraulic fracturing wastewater, Chem. Eng. J. (2021), https://doi.org/10.1016/j.cej.2021.131299.

[90] W.Y. Cheah, T.C. Ling, P.L. Show, J.C. Juan, J.S. Chang, D.J. Lee, Cultivation in wastewaters for energy: a microalgae platform, Appl. Energy (2016), https://doi.org/10.1016/j.apenergy.2016.07.015.

[91] D. Wang, Y.C. Lai, A.L. Karam, F.L. De Los Reyes, J.J. Ducoste, Dynamic modeling of microalgae growth and lipid production under transient light and nitrogen conditions, Environ. Sci. Technol. (2019), https://doi.org/10.1021/acs.est.9b02908.

[92] G.A. Lutzu, M.A. Marin, A. Concas, N.T. Dunford, Growing picochlorum oklahomensis in hydraulic fracturing wastewater supplemented with animal wastewater, Water Air. Soil Pollut. 231 (2020) 457, https://doi.org/10.1007/s11270-020-04826-1.

[93] X. You, L. Yang, X. Zhou, Y. Zhang, Sustainability and carbon neutrality trends for microalgae-based wastewater treatment: a review, Environ. Res. (2022), https://doi.org/10.1016/j.envres.2022.112860.

[94] R. Dineshkumar, A.S. Chauhan, R. Sen, Optimal and strategic delivery of CO_2 for *Chlorella minutissima* mediated valorization of domestic wastewater with concomitant production of biomass and biofuel, Sustain. Energy Fuels (2020), https://doi.org/10.1039/d0se00296h.

[95] Y. Li, W.A. Tarpeh, K.L. Nelson, T.J. Strathmann, Quantitative evaluation of an integrated system for valorization of wastewater algae as bio-oil, fuel gas, and fertilizer products, Environ. Sci. Technol. (2018), https://doi.org/10.1021/acs.est.8b04035.

[96] J.M. Do, S.W. Jo, H.T. Yeo, D.H. Shin, H. Oh, J.W. Hong, H.S. Yoon, Biological treatment of reverse osmosis concentrate by microalgae cultivation and utilization of the resulting algal biomass, J. Water Process Eng. (2021), https://doi.org/10.1016/j.jwpe.2021.102157.

[97] Y. Li, Y.F. Chen, P. Chen, M. Min, W. Zhou, B. Martinez, J. Zhu, R. Ruan, Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production, Bioresour. Technol. (2011), https://doi.org/10.1016/j.biortech.2011.01.091.

[98] H. Wang, H. Xiong, Z. Hui, X. Zeng, Mixotrophic cultivation of *Chlorella pyrenoidosa* with diluted primary piggery wastewater to produce lipids, Bioresour. Technol. (2012), https://doi.org/10.1016/j.biortech.2011.11.020.

[99] M.B. Johnson, Z. Wen, Development of an attached microalgal growth system for biofuel production, Appl. Microbiol. Biotechnol. (2010), https://doi.org/10.1007/s00253-009-2133-2.

[100] G. Hodaifa, M.E. Martínez, S. Sánchez, Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*, Bioresour. Technol. (2008), https://doi.org/10.1016/j.biortech.2007.02.020.

[101] G.A. Lutzu, W. Zhang, T. Liu, Feasibility of using brewery wastewater for biodiesel production and nutrient removal by *Scenedesmus dimorphus*, Environ. Technol. 37 (2016) 1568–1581.

[102] R.J. Wicker, E. Kwon, E. Khan, V. Kumar, A. Bhatnagar, The potential of mixed-species biofilms to address remaining challenges for economically-feasible microalgal biorefineries: a review, Chem. Eng. J. 451 (2023) 138481, https://doi.org/10.1016/j.cej.2022.138481.

[103] R.N. Singh, S. Sharma, Development of suitable photobioreactor for algae production–a review, Renew. Sustain. Energy Rev. 16 (2012) 2347–2353.

[104] K.K. Sharma, H. Schuhmann, P.M. Schenk, High lipid induction in microalgae for biodiesel production, Energies (2012), https://doi.org/10.3390/en5051532.

[105] A. Morillas-España, T. Lafarga, A. Sánchez-Zurano, F.G. Acién-Fernández, C. González-López, Microalgae based wastewater treatment coupled to the production of high value agricultural products: current needs and challenges, Chemosphere 291 (2022), https://doi.org/10.1016/j.chemosphere.2021.132968.

[106] I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux, Biodiesel from microalgae: a critical evaluation from laboratory to large scale production, Appl. Energy (2012).

[107] G. Policastro, A. Cesaro, M. Fabbricino, Photo-fermentative hydrogen production from cheese whey: engineering of a mixed culture process in a semi-continuous, tubular photo-bioreactor, Int. J. Hydrog. Energy 48 (2023) 21038–21054, https://doi.org/10.1016/j.ijhydene.2022.07.063.

[108] A. Al-Dailami, I. Koji, I. Ahmad, M. Goto, Potential of photobioreactors (PBRs) in cultivation of microalgae, J. Adv. Res. Appl. Sci. Eng. Technol. 27 (2022) 32–44, https://doi.org/10.37934/araset.27.1.3244.

[109] Q. Huang, F. Jiang, L. Wang, C. Yang, Design of photobioreactors for mass cultivation of photosynthetic organisms, Engineering (2017), https://doi.org/10.1016/J.ENG.2017.03.020.

[110] J. Huang, M. Wan, J. Jiang, A. Zhang, D. Zhang, Evaluating the effects of geometry and arrangement parameter of flat panel photobioreactor on microalgae biomass production and economic performance in China, Algal Res 57 (2021) 102343, https://doi.org/10.1016/j.algal.2021.102343.

[111] E. Segredo-Morales, E. González, C. González-Mart\'\in, L. Vera, Evaluation of membrane fouling in a microalgal-bacterial membrane photobioreactor treating secondary wastewater effluent: effect of photoperiod conditions, Environ. Sci. Water Res. Technol. 9 (2023) 1672–1682.

[112] J.H. Moreno Osorio, A. Pollio, L. Frunzo, P.N.L. Lens, G. Esposito, A review of microalgal biofilm technologies: definition, applications, settings and analysis, Front. Chem. Eng. (2021), https://doi.org/10.3389/fceng.2021.737710.

[113] J. Wang, W. Liu, T. Liu, Biofilm based attached cultivation technology for microalgal biorefineries—a review, Bioresour. Technol. (2017), https://doi.org/10.1016/j.biortech.2017.05.136.

[114] Y. Zhang, C. Ji, W. Zhou, H. Wang, J. Wang, T. Liu, Studies on the attached cultivation of filamentous oleaginous microalga *Tribonema minus*, J. Ocean Univ. China (2020), https://doi.org/10.1007/s11802-020-4256-0.

[115] L.B. Christenson, R.C. Sims, Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products, Biotechnol. Bioeng. (2012), https://doi.org/10.1002/bit.24451.

[116] Z. Mousavian, M. Safavi, A. Salehirad, F. Azizmohseni, M. Hadizadeh, S. Mirdamadi, Improving biomass and carbohydrate production of microalgae in the rotating cultivation system on natural carriers, AMB Express (2023), https://doi.org/10.1186/s13568-023-01548-5.

[117] L. Zhang, L. Chen, J. Wang, Y. Chen, X. Gao, Z. Zhang, T. Liu, Attached cultivation for improving the biomass productivity of *Spirulina platensis*, Bioresour. Technol. (2015), https://doi.org/10.1016/j.biortech.2015.01.025.

[118] H. Ennaceri, T. Ishika, V.O. Mkpuma, N.R. Moheimani, Microalgal biofilms: towards a sustainable biomass production, Algal Res (2023), https://doi.org/10.1016/j.algal.2023.103124.

[119] Y.K. Leong, J.S. Chang, D.J. Lee, Types of photobioreactors, Curr. Dev. Biotechnol. Bioeng. Photo Des. Appl. (2023), https://doi.org/10.1016/B978-0-323-99911-3.00007-5.

[120] K. Gaurav, K. Neeti, R. Singh, Microalgae-based biodiesel production and its challenges and future opportunities: a review, Green. Technol. Sustain. (2024), https://doi.org/10.1016/j.grets.2023.100060.

[121] A. Richmond, Handbook of microalgal culture: biotechnology and applied phycology, Wiley-Blackwell, 2008.

[122] R.H. Wijffels, M.J. Barbosa, An outlook on microalgal biofuels, Sci. (80-.) (2010), https://doi.org/10.1126/science.1189003.

[123] B.D. Fernandes, A. Mota, J.A. Teixeira, A.A. Vicente, Continuous cultivation of photosynthetic microorganisms: approaches, applications and future trends, Biotechnol. Adv. 33 (2015) 1228–1245, https://doi.org/10.1016/j.biotechadv.2015.03.004.

[124] G. Yadav, B.K. Dubey, R. Sen, A comparative life cycle assessment of microalgae production by CO_2 sequestration from flue gas in outdoor raceway ponds under batch and semi-continuous regime, J. Clean. Prod. 258 (2020) 120703, https://doi.org/10.1016/j.jclepro.2020.120703.

[125] S. Nagappan, S. Devendran, P.C. Tsai, H.U. Dahms, V.K. Ponnusamy, Potential of two-stage cultivation in microalgae biofuel production, Fuel 252 (2019) 339–349, https://doi.org/10.1016/j.fuel.2019.04.138.

[126] A. San Pedro, C.V. González-López, F.G. Acién, E. Molina-Grima, Marine microalgae selection and culture conditions optimization for biodiesel production, Bioresour. Technol. 134 (2013) 353–361, https://doi.org/10.1016/j.biortech.2013.02.032.

[127] A.N. Modenes, M. Zaharieva, H. Najdenski, Modeling and Technoeconomic Analysis of Algae for Bioenergy and Coproducts, Elsevier B.V, 2017, https://doi.org/10.1016/B978-0-444-63784-0.00011-4.

[128] N. Pragya, K.K. Pandey, P.K.K. Sahoo, A review on harvesting, oil extraction and biofuels production technologies from microalgae, Renew. Sustain. Energy Rev. 24 (2013) 159–171, https://doi.org/10.1016/J.RSER.2013.03.034.

[129] M. Hannon, J. Gimpel, M. Tran, B. Rasala, S. Mayfield, Biofuels from algae: challenges and potential, Biofuels 1 (2010) 763–784, https://doi.org/10.4155/bfs.10.44.

[130] S. van Dyk, J. Su, J.D. Mcmillan, J. (John Saddler, Potential synergies of drop-in biofuel production with further co-processing at oil refineries, Biofuels, Bioprod. Bioref. 13 (2019) 760–775, https://doi.org/10.1002/bbb.1974.

[131] P. Kurzawska, R. Jasiński, Overview of sustainable aviation fuels with emission characteristic and particles emission of the turbine engine fueled ATJ blends with different percentages of ATJ fuel, Energies 14 (2021), https://doi.org/10.3390/en14071858.

[132] IATA, 2024(accessed May 6, 2024)., Jet. Fuel Price Monitor .⟨https://www.iata.org/en/publications/economics/fuel-monitor/⟩(accessed May 6, 2024).

[133] J. Ruiz, G. Olivieri, J. De Vree, R. Bosma, P. Willems, J.H. Reith, M.H.M.M. Eppink, D.M.M.M. Kleinegris, R.H. Wijffels, M.J. Barbosa, 2016, , Towards industrial products from microalgae.

[134] M.R. Tredici, L. Rodolfi, N. Biondi, N. Bassi, G. Sampietro, Techno-economic analysis of microalgal biomass production in a 1-ha Green Wall Panel (GWP®) plant, Algal Res 19 (2016) 253–263, https://doi.org/10.1016/j.algal.2016.09.005.

[135] H. Jin, W. Chuai, K. Li, G. Hou, M. Wu, J. Chen, H. Wang, J. Jia, D. Han, Q. Hu, Ultrahigh-cell-density heterotrophic cultivation of the unicellular green alga *Chlorella sorokiniana* for biomass production, Biotechnol. Bioeng. 118 (2021) 4138–4151, https://doi.org/10.1002/BIT.27890.

[136] H. Jin, H. Zhang, Z. Zhou, K. Li, G. Hou, Q. Xu, W. Chuai, C. Zhang, D. Han, Q. Hu, Ultrahigh-cell-density heterotrophic cultivation of the unicellular green microalga *Scenedesmus acuminatus* and application of the cells to photoautotrophic culture enhance biomass and lipid production, Biotechnol. Bioeng. 117 (2020) 96–108, https://doi.org/10.1002/BIT.27190.

[137] R. Araújo, F. Vázquez Calderón, J. Sánchez López, I.C. Azevedo, A. Bruhn, S. Fluch, M. Garcia Tasende, F. Ghaderiardakani, T. Ilmjärv, M. Laurans, M. Mac Monagail, S. Mangini, C. Peteiro, C. Rebours, T. Stefansson, J. Ullmann, Current status of the algae production industry in Europe: an emerging sector of the blue

bioeconomy, Front. Mar. Sci. 7 (2021) 1–24, https://doi.org/10.3389/fmars.2020.626389.
[138] S. de Jong, J. van Stralen, M. Londo, R. Hoefnagels, A. Faaij, M. Junginger, Renewable jet fuel supply scenarios in the European Union in 2021–2030 in the context of proposed biofuel policy and competing biomass demand, GCB Bioenergy 10 (2018) 661–682, https://doi.org/10.1111/GCBB.12525.
[139] K.T. Wu, C.J. Tsai, C.S. Chen, H.W. Chen, The characteristics of torrefied microalgae, Appl. Energy 100 (2012) 52–57, https://doi.org/10.1016/J.APENERGY.2012.03.002.
[140] K.S. Ng, D. Farooq, A. Yang, Global biorenewable development strategies for sustainable aviation fuel production, Renew. Sustain. Energy Rev. 150 (2021) 111502, https://doi.org/10.1016/J.RSER.2021.111502.
[141] O.S. Djandja, Z. Wang, L. Chen, L. Qin, F. Wang, Y. Xu, P. Duan, Progress in hydrothermal liquefaction of algal biomass and hydrothermal upgrading of the subsequent crude bio-oil: a mini review, Energy Fuels 34 (2020) 11723–11751, https://doi.org/10.1021/ACS.ENERGYFUELS.0C01973/ASSET/IMAGES/LARGE/EF0C01973_0008.JPEG.
[142] P. Ranganathan, S. Savithri, Techno-economic analysis of microalgae-based liquid fuels production from wastewater via hydrothermal liquefaction and hydroprocessing, Bioresour. Technol. 284 (2019) 256–265, https://doi.org/10.1016/J.BIORTECH.2019.03.087.
[143] J. Roles, J. Yarnold, K. Hussey, B. Hankamer, Techno-economic evaluation of microalgae high-density liquid fuel production at 12 international locations, Biotechnol. Biofuels 14 (2021) 1–19, https://doi.org/10.1186/S13068-021-01972-4/FIGURES/4.
[144] M.L.N.M.M. Carneiro, F. Pradelle, S.L. Braga, M.S.P. Gomes, A.R.F.A.A. Martins, F. Turkovics, R.N.C.C. Pradelle, Potential of biofuels from algae: comparison with fossil fuels, ethanol and biodiesel in Europe and Brazil through life cycle assessment (LCA), Renew. Sustain. Energy Rev. 73 (2017) 632–653, https://doi.org/10.1016/J.RSER.2017.01.152.
[145] N.D. Orfield, R.B. Levine, G.A. Keoleian, S.A. Miller, P.E. Savage, Growing algae for biodiesel on direct sunlight or sugars: a comparative life cycle assessment, ACS Sustain. Chem. Eng. 3 (2015) 386–395, https://doi.org/10.1021/SC5004117/SUPPL_FILE/SC5004117_SI_001.PDF.
[146] E.P. Bennion, D.M. Ginosar, J. Moses, F. Agblevor, J.C. Quinn, Lifecycle assessment of microalgae to biofuel: comparison of thermochemical processing pathways, Appl. Energy 154 (2015) 1062–1071, https://doi.org/10.1016/J.APENERGY.2014.12.009.
[147] M. Prussi, W. Weindorf, M. Buffi, J. Sánchez López, N. Scarlat, Are algae ready to take off? GHG emission savings of algae-to-kerosene production, Appl. Energy 304 (2021) 117817, https://doi.org/10.1016/J.APENERGY.2021.117817.
[148] H.H.H. Khoo, P.N.N. Sharratt, P. Das, R.K.K. Balasubramanian, P.K. K. Naraharisetti, S. Shaik, Life cycle energy and CO_2 analysis of microalgae-to-biodiesel: Preliminary results and comparisons, Bioresour. Technol. 102 (2011) 5800–5807, https://doi.org/10.1016/J.BIORTECH.2011.02.055.
[149] Y.K. Dasan, M.K. Lam, S. Yusup, J.W. Lim, K.T. Lee, Life cycle evaluation of microalgae biofuels production: effect of cultivation system on energy, carbon emission and cost balance analysis, Sci. Total Environ. 688 (2019) 112–128, https://doi.org/10.1016/J.SCITOTENV.2019.06.181.
[150] R. Huang, J. Li, Y. Tang, W. Song, Y. Yu, W. Yang, J. Cheng, Comparative life-cycle assessment of microalgal biodiesel production via various emerging wet scenarios: energy conversion characteristics and environmental impacts, Energy Convers. Manag. 257 (2022) 115427, https://doi.org/10.1016/J.ENCONMAN.2022.115427.
[151] A. Weiss, L. Schebek, The net energy ratio of microalgae biofuels production based on correlated cultivation parameters in flat plate photobioreactors, J. Clean. Prod. 287 (2021) 125073, https://doi.org/10.1016/J.JCLEPRO.2020.125073.
[152] F.G. Acién, J.M. Fernández, J.J. Magán, E. Molina, Production cost of a real microalgae production plant and strategies to reduce it, Biotechnol. Adv. 30 (2012) 1344–1353, https://doi.org/10.1016/j.biotechadv.2012.02.005.
[153] P. Pérez-López, J.H. de Vree, G. Feijoo, R. Bosma, M.J. Barbosa, M.T. Moreira, R. H. Wijffels, A.J.B. van Boxtel, D.M.M. Kleinegris, Comparative life cycle assessment of real pilot reactors for microalgae cultivation in different seasons, Appl. Energy 205 (2017) 1151–1164, https://doi.org/10.1016/j.apenergy.2017.08.102.
[154] M. Guiton, D. Suárez-Montes, R. Sánchez, P. Baustert, C. Soukoulis, B.S. Okan, T. Serchi, S. Cambier, E. Benetto, Comparative life cycle assessment of a microalgae-based oil metal working fluid with its petroleum-based and vegetable-based counterparts, J. Clean. Prod. 338 (2022) 130506, https://doi.org/10.1016/J.JCLEPRO.2022.130506.
[155] M.K. Mediboyina, B.K. Banuvalli, V.S. Chauhan, S.N. Mudliar, Comparative life cycle assessment of autotrophic cultivation of *Scenedesmus dimorphus* in raceway pond coupled to biodiesel and biogas production, Bioprocess Biosyst. Eng. 43 (2020) 233–247, https://doi.org/10.1007/S00449-019-02220-8/FIGURES/8.
[156] M.R. Tredici, N. Bassi, M. Prussi, N. Biondi, L. Rodolfi, G. Chini Zittelli, G. Sampietro, Energy balance of algal biomass production in a 1-ha "Green Wall Panel" plant: how to produce algal biomass in a closed reactor achieving a high Net Energy Ratio, Appl. Energy 154 (2015) 1103–1111, https://doi.org/10.1016/J.APENERGY.2015.01.086.
[157] I.B. Magalhães, J. Ferreira, J. de S. Castro, L.R. de Assis, M.L. Calijuri, Agro-industrial wastewater-grown microalgae: a techno-environmental assessment of open and closed systems, Sci. Total Environ. 834 (2022) 155282, https://doi.org/10.1016/J.SCITOTENV.2022.155282.
[158] P.K. Campbell, T. Beer, D. Batten, Life cycle assessment of biodiesel production from microalgae in ponds, Bioresour. Technol. 102 (2011) 50–56, https://doi.org/10.1016/J.BIORTECH.2010.06.048.
[159] V. Heredia, J. Legrand, J. Pruvost, Microalgal biofuels: pathways towards a positive energy balance, Energy Convers. Manag. 267 (2022) 115929, https://doi.org/10.1016/J.ENCONMAN.2022.115929.
[160] S. Schade, T. Meier, A comparative analysis of the environmental impacts of cultivating microalgae in different production systems and climatic zones: a systematic review and meta-analysis, Algal Res 40 (2019) 101485, https://doi.org/10.1016/j.algal.2019.101485.
[161] M. Collotta, L. Busi, P. Champagne, W. Mabee, G. Tomasoni, M. Alberti, Evaluating microalgae-to-energy -systems: different approaches to life cycle assessment (LCA) studies, Biofuels, Bioprod. Bioref. 10 (2016) 883–895, https://doi.org/10.1002/BBB.1713.
[162] S. De Jong, K. Antonissen, R. Hoefnagels, L. Lonza, M. Wang, A. Faaij, M. Junginger, Life-cycle analysis of greenhouse gas emissions from renewable jet fuel production, Biotechnol. Biofuels 10 (2017) 1–18, https://doi.org/10.1186/S13068-017-0739-7/TABLES/4.
[163] Q. Tu, M. Eckelman, J. Zimmerman, Meta-analysis and harmonization of life cycle assessment studies for algae biofuels, Environ. Sci. Technol. 51 (2017) 9419–9432, https://doi.org/10.1021/ACS.EST.7B01049/SUPPL_FILE/ES7B01049_SI_001.PDF.
[164] C. Quiroz Arita, Ö. Yilmaz, S. Barlak, K.B. Catton, J.C. Quinn, T.H. Bradley, A geographical assessment of vegetation carbon stocks and greenhouse gas emissions on potential microalgae-based biofuel facilities in the United States, Bioresour. Technol. 221 (2016) 270–275, https://doi.org/10.1016/J.BIORTECH.2016.09.006.
[165] R. Usami, K. Fujii, C. Fushimi, Improvement of bio-oil and nitrogen recovery from microalgae using two-stage hydrothermal liquefaction with solid carbon and HCl acid catalysis, ACS Omega 5 (2020) 6684–6696, https://doi.org/10.1021/ACSOMEGA.9B04468/ASSET/IMAGES/LARGE/AO9B04468_0002.JPEG.