Chemical Engineering Journal 451 (2023) 138481

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Review

The potential of mixed-species biofilms to address remaining challenges for economically-feasible microalgal biorefineries: A review

Rebecca J. Wicker [a,*], Eilhann Kwon [b], Eakalak Khan [c], Vinod Kumar [d], Amit Bhatnagar [a]

[a] Department of Separation Science, LUT School of Engineering Science, LUT University, Sammonkatu 12, 50130 Mikkeli, Finland
[b] Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul 04763, Republic of Korea
[c] Department of Civil and Environmental Engineering and Construction, University of Nevada, Las Vegas 89154 USA
[d] School of Water, Energy and Environment, Cranfield University, Cranfield MK43 0AL, UK

ARTICLE INFO

Keywords:
Microalgal-bacterial consortia
Biofilms
Attached cultivation
Environmental biotechnology
Bioenergy

ABSTRACT

Several key challenges are hindering large-scale cultivation of microalgae for industrial purposes, including wastewater treatment, carbon capture, biomass production, and renewable energy production. These challenges are closely related to efficacy of 1) resource utilization, 2) biomass production, and 3) harvesting. This review describes how attached or biofilm cultivation of microalgae and/or cyanobacteria with heterotrophic bacteria in consortia could simultaneously resolve these technical obstacles, thereby reducing monetary and energetic costs of producing microalgal bioenergy. Symbiotic relationships between these organisms reduces the need for aeration or exogenous supplementation of nutrients. Additionally, this review details how increasing biodiversity correlates with diversity of functionality (carbon capture and nitrification) and how attached/biofilm cultivation can improve photosynthetic efficiency and water footprint. Mixed-species biofilms have persisted for billions of years across earth's natural history because they are some of nature's most highly efficient biosystems, and they deserve more dedicated study and broader application in bioenergy production. This review details the practical connections between microalgal-bacterial consortia, attached/biofilm cultivation, waste-to-value biorefining, and relevance to bioenergy production and value-added products (VAPs); four topics previously unconnected in a single review. As such this review aims to bridge current knowledge gaps across multiple research fields and industrial sectors, towards the goal of efficient, economical, and climate-forward microalgal bio-services and bioenergy production.

1. Introduction

Microalgae cultivation has often been heralded as a renewable/sustainable answer to numerous modern challenges, especially that of carbon–neutral or -negative bioenergy and biofuels. A highly diverse group of photosynthetic microorganisms, the umbrella term "microalgae" may describe eukaryotic green algae, diatoms, and protists, as well as prokaryotic cyanobacteria. Microalgae may be single-celled and pelagic, or filamentous/colonial, forming mats or biofilms. Microalgae utilize carbon dioxide (CO_2) at a rate 10–50 times greater than that of land plants [1], and they produce biomass much faster than their terrestrial counterparts [2]. They are well known to capture nutrients (nitrogen and phosphorus) which can trigger eutrophication in natural waterways [3–7], and have shown additional promise in removing other environmentally damaging compounds, such as heavy metals [8,9], and emergent pollutants, such as pharmaceuticals [10,11]. Their aptitude for assimilating CO_2 and recycling organic waste into biomass and oxygen has even made them a biotechnological candidate for facilitating long-term human survival in space [12,13]. There remain several technical obstacles in large-scale microalgae cultivation, however, hindering their widespread application in various industries, especially regarding production of bioenergy and biofuels. Microalgal growth is limited by different factors such as light intensity, nutrient availability, aggregation, and intolerance to extreme temperatures and pH [14]. The species most commonly sought in industrial applications are pelagic, or free-living; harvesting these small microalgal cells in relatively disperse liquid cultures is expensive and energy-intensive, and downstream processes such as lipid extraction for biodiesel production can inflate these costs significantly [15,16], sometimes outweighing the net monetary or energetic yield of the finished products entirely [17,18].

* Corresponding author.
E-mail address: rebecca.wicker@lut.fi (R.J. Wicker).

https://doi.org/10.1016/j.cej.2022.138481
Received 6 June 2022; Received in revised form 16 July 2022; Accepted 2 August 2022
Available online 4 August 2022

Microalgae alone cannot provide viable solutions to these challenges.

Microalgae, however, did not evolve in vacuum. The eukaryotic single-celled green microalgae species that dominate most microalgal technology initiatives were not even the first microorganisms to photosynthesize [19]. Phototropic bacteria preceded all photosynthetic life on the planet today, and, during the course of Earth's natural history, microalgae, cyanobacteria, and an enormous variety of other microbes have competed, cooperated, and communicated within interconnected micro-universes ubiquitous across the planet. These micro-universes are termed "phycospheres" [20]. Co-culture of phytoplankton (eukaryotic microalgae, diatoms, and protists, as well as prokaryotic cyanobacteria) with non-photosynthetic bacteria can address a number of problems associated with microalgal monoculture, especially with regards to harvesting efficiency and resource utilization. Multiple recent studies have demonstrated that co-culture of phytoplankton along with heterotrophic bacteria is beneficial to both groups [21–23]. Bacteria evolve CO_2, which is used as a substrate for photosynthesis, while photosynthesis provides the oxygen required for bacterial and/or dark microalgal oxidation of compounds such as ammonium and nitrite, as well as degradation of organic carbon species [24,25]. Synergistic interactions have been observed between both groups when they are used in concert for wastewater treatment [22], and co-culture has shown promise in reducing overall GHG emissions from waste treatment processes [26]. Specific to the challenge of efficient harvesting, a well-established technology for pollution remediation, called the Algal Turf Scrubber (ATS) system, has been developed for attached cultivation of indigenous phytoplankton [27]. Previously deployed for nutrient pollution remediation in natural water systems [28], the ATS system can be modified for use in various types of bioreactor systems. Encouraging attached growth of phytoplankton has been shown to enhance biomass production and nutrient removal [6], and significantly increase harvesting efficiency [29]. Finally, as co-culture can substantially increase biomass while simultaneously reducing the costs and energy requirements of cultivation and harvesting, it increases the net value of finished products, and can diversify the product output [30,31]. The added value of microalgal-bacterial co-culture in attached or biofilm cultivation modes has strong potential to address several key challenges affecting the world today.

The aim of this review is to demonstrate that, in order to fully exploit the advantages of photosynthetic mixed-species biofilms for industrially-relevant production of bioenergy and value-added products, cultivation systems must be redesigned with ecological principles in mind. Mixed-species biofilms are comprised of multiple microorganism species (eukaryotic microalgae, cyanobacteria, and/or heterotrophic bacteria) immobilized within a biological matrix exuded by one or more biofilm member species. This review explores the fundamental biology of a concept not yet widely investigated in research; combining the advantages of attached and biofilm cultivation with mixed-species consortia (in lieu of conventional monoculture) (Table 1) towards the goal of economically-competitive microalgal biofuels and bioenergy products. These numerous, concomitant advantages are thoroughly detailed herein. Using nature as a model, next-generation photobioreactors (PBRs) should strive to emulate the microbial mats and biofilms that have persisted for virtually all of earth's known natural history. By capitalizing upon natural mutualistic and synergistic interspecies interactions and creating robust, resilient microalgal consortia, the full potential of photosynthetic mixed-species biofilms can be realized on the industrial scale.

2. Obstacles remaining in microalgal bioenergy production

Cultivating microalgae on the industrial scale is technologically and economically challenging, and these issues must be resolved before microalgal biomass can be implemented as a commercially-viable bioenergy feedstock. Most current bottlenecks in large-scale cultivation of microalgae are ascribed to high-volume cultures with low cell density [32]. Low cell is the primary cause of three main obstacles; slow nutrient uptake, low biomass production, and inefficient biomass harvesting. In these disperse cultures, nutrient uptake and substrate exchange are limited, and biomass yields are reduced by less-than-optimum biomass production and difficulties associated with harvesting. Furthermore, the energy requirements of cell concentration and dewatering can inflate the cost of biomass, reducing the economic competitiveness of resultant bioproducts. Considering their ability to grow in nutrient-dense wastewater, and the fact that they generate oxygen via photosynthesis whilst consuming carbon dioxide [33], microalgae have been considered as a promising new biotechnology for wastewater treatment [23]. Microalgal bioenergy products, however, will not become economically viable or competitive until these efficiency obstacles are overcome.

2.1. Nutrient uptake efficiency

Considering microalgal cultivation as an eco-friendly wastewater treatment coupled to bioenergy production, efficiency of nutrient removal is an important factor. The first major difficulty is the sheer volume of wastewater produced from various industrial processes. Nutrient-rich wastewater is frequently discharged to the environment with inadequate treatment, leading to ecological catastrophes such as eutrophication and widespread deoxygenation of natural ecosystems [34]. Eutrophication and deoxygenation events are not only devastating to wildlife and the natural environment, but should also be considered a severe public health concern, as some of the microorganisms involved are known to produce potent toxins [34]. Microalgae have long been considered a strong candidate for biological treatment of wastewater, but are not yet widely implemented on the industrial scale. One major drawback is the very low cell density of suspended (liquid media) cultivation. In disperse liquid cultures, biomass content may be as low as 0.1–0.5 % dry mass. Diffusion processes (i.e. the transmission of molecules between the environment and organisms) dependent upon nutrient concentration may be too slow to facilitate efficient mass transfer and support biological demands for economically-efficient growth [32]. Slow diffusion of nutrients to biomass results in long

Table 1
Comparison between topics detailed in the present review and other recently published reviews (≤5 years) on mixed-species consortia and attached/biofilm cultivation modes.

Eukaryotic-prokaryotic interactions	Attached/biofilm cultivation	O_2/carbon exchange	Nutrient recovery	Biomass production	Harvesting efficiency	Photobioreactor design	References
✓	✓	✓	✓	✓	✓	✓	This review
✓	✓	×	×	×	×	×	[46]
×	✓	×	×	✓	✓	✓	[32]
✓	✓	×	✓	✓	✓	✓	[34]
×	✓	✓	×	✓	✓	✓	[35]
✓	×	✓	✓	✓	✓	×	[48]
✓	✓	✓	✓	✓	×	✓	[24]
✓	×	✓	✓	✓	×	×	[55]
✓	✓	×	×	✓	✓	✓	[91]

hydraulic retention times which cannot keep up with the rate of wastewater production [35,36], thus disqualifying microalgal biotreatment of wastewater as an industrially viable option at present, especially with the ultimate goal of producing bioenergy from the biomass generated. Furthermore, other substances common in wastewater (e.g. heavy metals, pharmaceuticals, or other toxic pollutants) can inhibit nutrient uptake by microalgae, and negatively impact growth and bioproduction [9,10].

2.2. Biomass production efficiency

Economically efficient biomass productivity is intricately linked with nutrient uptake and resource utilization. If diffusion and mass transfer are suboptimal, the doubling time and overall growth rate of microalgal cells in suspension will likewise fall below the maximum possible biological potential, thereby impacting economic returns and product yields from biomass [32]. Although conventional suspended microalgal culture techniques still dominate in the industry, this cultivation mode cannot produce biomass efficiently enough to generate economically competitive bioenergy products. The only currently economically-viable microalgal bioproducts produced on the industrial scale are the absolute highest market value compounds, primarily food-grade health supplements. Factors that limit the growth rate of microalgae in suspended cultures include light penetration, nutrient dispersal, and varying levels of gas mass transfer throughout the media [37]. Even in well-mixed systems, microalgal cells are prone to self-shading (restricting effective light utilization) [38] and oxygen inhibition of photosynthesis once the culture reaches a threshold density [39]. To reduce the cost of microalgal bioenergy products, the net biomass generated per liter of cultivation medium must be increased significantly.

2.3. Microalgal harvesting efficiency

Even in the most productive suspension cultures, microalgal harvesting efficiency remains a severe bottleneck. Several species of pelagic microalgae (such as *Chlorella vulgaris* and *Haematococcus pluvialis*) and cyanobacteria (such as *Arthrospira platensis*, commercially known as "Spirulina") are cultivated on the industrial scale for their high market value bioactive compounds (proteins, pigments, and omega-3 fatty acids). However, harvesting costs are still far too high to be compensated by the sale of any other extractable compound [37]. The cell densities of suspended cultures generally range from 0.5 to 5 g dry biomass L^{-1}, and cell sizes tend to range from 5 to 20 μm, and such facts make harvesting processes complex and energetically expensive. The most common bulk harvesting technique applied at the industrial scale is centrifugation, which, although relatively efficient, is often prohibitively expensive in terms of capital costs and high energy requirements for operation [40]. Considering the total cost of biofuel production from microalgae, harvesting accounts for 20–30 % [41,42]. Another physical harvesting technique common in wastewater treatment and applicable to microalgal harvesting is membrane technology. Although membranes can be highly efficient, the negatively charged particles and polymers (such as microalgal cells and associated extracellular polymeric substances) can exacerbate membrane fouling [43]. Additionally, membrane separation requires high fluid pressure and energy expenditure, which can incur prohibitive operational costs over time [44].

Such significant expense raises operational costs enough that the whole biofuel process chain is simply not cost-effective enough to make the finished biofuel products economically competitive with their fossil counterparts [45]. Cheaper harvesting techniques commonly employed in wastewater treatment, such as chemical coagulation and flocculation have been applied to microalgal suspension cultures in hopes of reducing operational costs, but these methods come with other drawbacks, such as contamination of biomass with heavy metals. Chemically contaminated biomass can severely restrict the applicability of upgrading pathways (from biomass to bioenergy products). Heavy metal toxicity can inhibit biological upgrading pathways, such as fermentation or anaerobic digestion of biomass, whereas the presence of heavy metals can complicate chemical upgrading pathways, such as transesterification. To date, there are no mature harvesting technologies that can balance efficiency and economics, such that microalgal biomass is a valuable feedstock for anything other than the highest value human health supplements [40]. Other low-cost, "greener" harvesting methods have been explored with some success; for example, bioflocculation with bio-based polymers or other organisms. Bio-based polymers (such as chitosan) tend to perform poorly unless they are augmented to carry a strong positive charge [46]. Amongst organisms used for flocculation, filamentous fungi and cyanobacteria have shown promise. Fungal and cyanobacterial filaments form a biological matrix which aggregates microalgal cells [40,47]. While this harvesting practice is eco-friendly and low cost, it can be slow and require a large amount of secondary biomass (e.g. fungi) to efficiently aggregate disperse microalgal cells. Attached and/or biofilm cultivation operates upon the same principle of biological aggregation, without the waiting time of the flocculation process.

3. Advantages of mixed-species consortia, biofilms, and attached cultivation

Axenic cultures, common in research, are pure monocultures of a single species, with no contaminant species like bacteria or protist grazers. They are necessary for many scientific procedures, such as genome sequencing, identifying products of a specific bioactive compound, or omics studies on interspecies relationships [48]. Despite having several important roles in research and industry, however, axenic cultures do not exist in natural systems, and are extremely difficult to establish and maintain in engineered systems. Some bacterial species are associated with extracellular polymeric substances (EPS) excreted by microalgae, and can be near impossible to remove from the culture [49]. Methods of strain isolation such as vortexing, sonication, and surfactant treatments can cause physical damage and cell death to the target species, resulting in weak axenic cultures. Antibiotic treatments can be as toxic to microalgae as they are to unwanted bacteria [48]. Currently, most industry-scale microalgal cultivation is focused towards high-cost cultivation of monocultures in closed PBR systems for a single high-value bioproduct; for example, *Haematococcus pluvialis* and astaxanthin [37]. However, considering the shortcomings (discussed in Section 2), suspension-based monocultures are not nearly efficient enough to facilitate production of economically-feasible biofuels or bioenergy products, such as ethanol. The answer to the questions of efficient nutrient removal, biomass production, and harvesting is attached and/or biofilm cultivation of mixed photosynthetic and non-photosynthetic consortia.

More than a century ago, the very first microalgal cultivars were established as biofilms [32]. Many species of pelagic (free-living) microalgae have a natural ability to immobilize themselves by adhering to surfaces, thus forming biofilms or filaments [34]. The foundation for microalgal biofilms is a hydrated polymeric matrix formed by EPS, secreted by both microalgal and bacterial consortium partners (Fig. 1). Living cell-to-cell within this biological matrix, exchange of nutrients and gases occurs at a much faster rate than in disperse liquid cultures, and the EPS matrix can retain exuded enzymes which catalyze nutrient uptake and other metabolic functions. Maximal surface area additionally allows for much greater photosynthetic efficiency; ultimately creating a highly bioactive micro-environment [50].

Mixed-species consortia are dynamic, and able to adapt as a community to changing environmental conditions [37,50,51]. When cultured in concert, diverse species establish a natural equilibrium, filling all available ecological niches within the system; this is known as the productivity-diversity relationship in biological systems. Species richness within a biofilm can facilitate complementary resource utilization, which maximizes biological production [52]. Additionally,

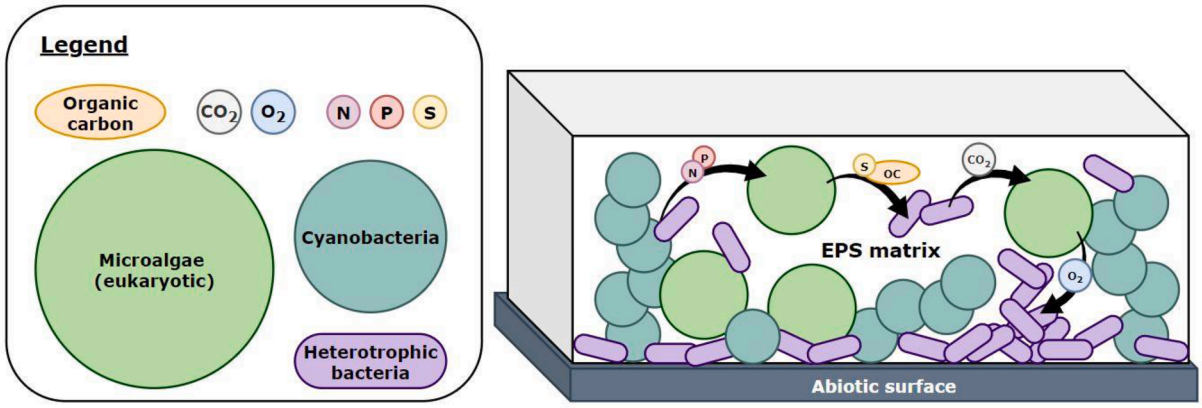

Fig. 1. Structure and substrate exchange within a mixed species biofilm (EPS, extracellular polymeric substances; N, nitrogen; P, phosphorus; S, sulfur; OC, organic carbon).

according to the ecological theory of alternate equilibria, biodiversity in a biological system is positively correlated with resilience to abrupt changes in conditions [52]. Depending on the wastewater treatment system, changes in pH, temperature, and water chemistry can be quite common, and cultivating an adaptive consortium can be highly advantageous. It is therefore important to pursue an understanding of positive interactions between highly diverse consortia members, such that interactions detrimental to bioproduction (including parasitism or grazing) can be minimized. Metagenomics is a highly useful tool for determining the species or genera present in a natural biofilm, and transcriptomics should be applied to ascertain the functional genes active under different environmental conditions. Using these two techniques, pre-adapted biofilm-forming species can be selected from different environments (such as wastewater treatment plants or pollution zones), and co-cultured together in a complementary fashion to achieve optimum resource use and bioproductivity.

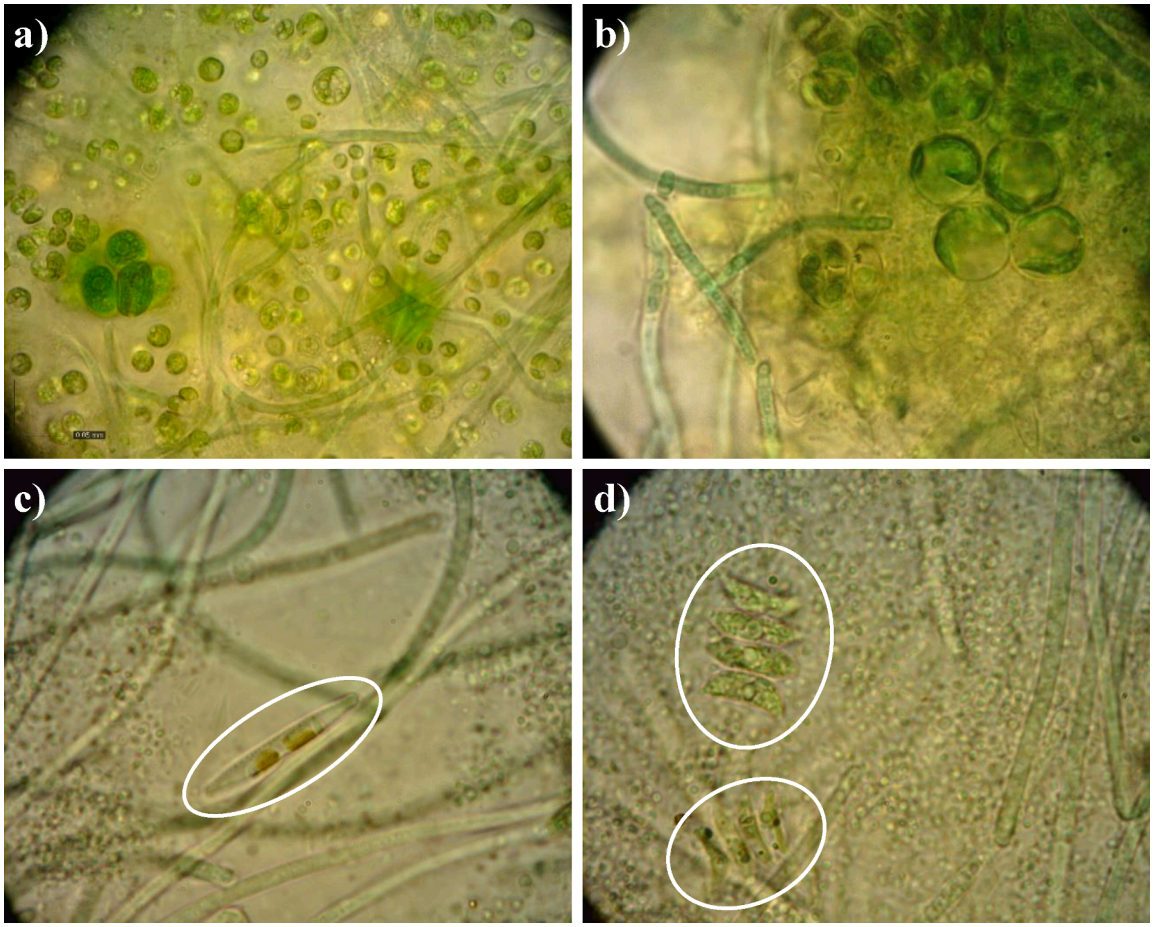

Fig. 2. Microscope images of different types of biofilms; a) *Chlamydomonas* sp. and other eukaryotes within a mixed cyanobacteria-heterotrophic bacteria biofilm, b) unidentified eukaryotes within a mixed cyanobacteria-heterotrophic bacteria biofilm, c) a diatom (circled) within a cyanobacterial biofilm, d) *Scenedesmus* sp. (circled) within a cyanobacterial biofilm.

3.1. Mutualistic interactions within biofilms and attached cultures

Within an ecological niche, interspecies interactions between different community members and balance the ecosystem as a whole. Interactions may be obligate or facultative [53]. An obligate relationship means that at least one species cannot survive without another. In a facultative relationship, one species alters their behavior or metabolism in order to interact with another species [49]. Symbiotic relationships describe interactions which are positive for at least one partner, although they may be detrimental as well, such as the case of parasitism. In a bioreactor environment, it is of paramount importance to encourage the most positive (and therefore productive) types of symbiosis between consortia members, such as mutualism and commensalism. These interactions may occur naturally [49] once species are cultivated in the same reactor, or they may need to be induced by controlling parameters such as light, temperature, pH, oxygenation, carbon speciation, and nutrient depletion or balance.

Symbiotic mutualism occurs when two or more species coexist in close proximity in an environment, with each species creating benefit for the other in some fashion. Benefits may include nutrient supply, gas exchange, protection, or even habitat creation, as in the case of lichen [53] or coral. Habitat creation occurs between microorganisms when filamentous or colonial species form a biofilm, in which otherwise pelagic organisms can grow physically attached (Fig. 2). In a mutualistic relationship, this attached co-cultivation increases the efficacy of nutrient and gas exchange by facilitating direct transfer of molecules rather than dispersal and reuptake. Symbiotic commensalism occurs when one partner benefits from others, but the relationship is neither harmful nor beneficial towards the other species involved, which are therefore considered “non-interacting” partners. Mutualism, rather than commensalism, is the optimal condition for bioproduction, as it most efficiently utilizes resources and enhances the growth of all partners involved, maximizing productive potential. It is important to select for positive interactions by combining cooperative species with environmental parameters that induce mutualistic interactions, as consortium members may also be antagonistic to one another. These factors must be optimized with targeted studies of interspecies interactions within mixed-species biofilms, from transcriptomic analyses to manipulating bioreactor conditions.

3.2. Substrate exchange

Microalgal growth and bioproduction require sufficient nutrients supplied in the growth media, such as nitrogen and phosphorus. Because these nutrients are expensive and potentially unsustainable (i.e. phosphorus mineral mining) [54], many research endeavors have utilized nutrient-rich wastewater streams as growth media [55,56]. While this approach is beneficial environmentally and economically, the nutrient composition of wastewaters from different industrial sources can vary substantially. Ratios of carbon and nitrogen are particularly important for microalgal cultivation. The obstacle of supplying reliable, sustainable nutrients to microalgal growth media could be partially overcome by synergistic interactions within microalgal-bacterial biofilms [41]. Dynamic consortia are able to degrade or adsorb complex pollutants [10,57], and different species with different metabolic functions can supply nutrients and substrates to other consortia members [58].

While many types of heterotrophic bacteria can form associations with microalgae, aerobic nutrient-oxidizing bacteria (such as nitrogen oxidizers) are the most abundant by far, as they require electrons from oxygen to oxidize organic carbon and nutrients such as ammonium and sulfur compounds in wastewater. Whereas wastewater can provide carbon and nutrients, a diverse microbial population is required to balance oxidation and reduction reactions which sustain the entire community. Bacterial nitrification is an important example of this balance; microalgae can assimilate nitrogen, but bacteria are the dominant organisms facilitating nitrogen cycling. Under aerobic conditions (as in a photosynthetic biofilm), ammonium is oxidized to nitrite, and then again to nitrate by bacteria before microalgae can effectively assimilate it [31].

A well-constrained example of symbiotic substrate exchange between microorganisms is the exchange of vitamin B12 and fixed carbon species between heterotrophic bacteria and eukaryotic microalgae [59]. Microalgae, like most of the plant kingdom, cannot independently produce vitamin B12, although it is an essential micronutrient, and necessary for effective growth and bioproduction. Likewise, many heterotrophic bacteria require a source of organic (fixed) carbon, as they cannot utilize inorganic species such as CO_2. Microalgae can provide a portion of this carbon requirement by excreting it into the media, most notably in the form of extracellular polymeric substances (EPS) which are exuded by eukaryotic microalgae and diatoms to protect the cell and its components [60]. EPS can also promote bacterial growth by storing nutrients so that they are readily available [61]. EPS and other organic material exuded by microalgae are known to cause chemotaxis in motile bacteria [20]. EPS accumulate around the microalgal cell and can provide a micro-habitat for bacterial cells, where they can attach and exchange nutrients easily, facilitating sufficient bacterial growth for microalgae to meet their vitamin B12 requirement in turn. Other types of fixed carbon sources provided by microalgae to bacteria include glycolate, a water-soluble two-carbon byproduct of photorespiration [60], and more complex compounds such as sulfonates, which simultaneously provide a source of carbon and other micronutrients (sulfur) needed by other bacterial species [53].

Another example of interspecies symbiosis is nutrient balance. Many cyanobacteria and heterotrophic bacteria can undergo dissimilatory nitrate reduction to ammonium, which is much more biologically available to eukaryotic green algae and diatoms [62]. Watari et al. [58] reported long-term stable nitrification by co-cultivating *Chlorella vulgaris* with aerobic sludge in a 6-chamber baffled photobioreactor. Over an extended cultivation period of 350 d, an average of 66 ± 11 % ammonia removal was achieved, with a nitrogen loading rate of 0.083 ± 0.011 kg m^{-3} day^{-1}. Their findings informed the establishment of community equilibrium over space and time, with the bacterial composition changing with the reactor and cultivation time. They found that, in terms of reactor space, the population of ammonia-oxidizing bacteria increased in the downstream chambers. In terms of time, after 200 d, nitrite-oxidizing bacteria populations began to fall due to competition with *C. vulgaris*. It was concluded that microalgae-nitrifying bacteria co-culture are potentially valuable as a partial nitrification bioprocess via combined photosynthetic activity and anammox [58]. Furthermore, bacteria solubilize phosphorus and iron, which are necessary for effective microalgal growth and bioproduction [53]. Balancing these nutrient cycles is important in establishing an artificial ecosystem within bioreactors for synergistic waste management and production of energy feedstock biomass.

The balance between oxygen (O_2) and CO_2 for efficient microalgal bioproduction could be delicate, as excess dissolved O_2 in medium can inhibit oxygenic photosynthesis. Additionally, oxygen bubbles may adhere to surfaces used for biofilm cultivation and can have mixed effects on characteristics of the biofilm; such as porosity, light penetration, and transfer of carbon and nutrients [63]. However, a recent study demonstrated that microalgal oxygen production enhanced bacterial activity in a dual-phase biofilm/sludge system for wastewater treatment [61]. This research demonstrated that, compared to conventional sequencing batch reactors, the removal rates of NO_2^-, NO_3^-, and PO_4^{3-} were increased by 43.7, 36.0, and 34.1 % respectively, in the sludge phase (where oxygenic photosynthesis was reduced). In the biofilm phase, however, removal rates of NO_2^-, NO_3^-, and PO_4^{3-} were enhanced by 174.0, 147.4, and 150.4 %, respectively. The authors attribute these remarkable findings to increases in active transport and uptake of divalent cations facilitated by favorable conditions within the EPS matrix, which can partially neutralize molecular charges [61]. Additionally, nutrient storage within the EPS that allowed microorganisms to use them as

needed, so that no extra energy was devoted to processes like luxury phosphorus uptake [61].

3.3. Extracellular signaling

Microorganisms of all types are known to secrete compounds with various functions, which may promote or inhibit the growth of other organisms. Signaling molecules such as indole-3 acetic acid (IAA), *N*-acyl-homoserine lactones (AHLs), and autoinducing peptides (AIPs) are well-known in microbial ecology, as they regulate physiological behaviors and gene expression in bacteria responsible for facilitating a wide variety of biogeochemical cycles[31]. This type of cell-to-cell communication is called quorum sensing, and its occurrence is closely related with population densities [31]. Quorum sensing molecules (QSM) have demonstrable value in microalgal cultivation. Between microalgae and bacteria, three general types of QSM have been documented; lipid-based molecules, bacterial signaling molecules (e.g. AHLs), and microalgal allelochemicals (e.g. flavonoids) [64]. Lipid-based signaling molecules are capable of energy-independent passage through cell membranes, triggering quick responses [64]. Molecules such as AHLs and allelochemicals are important in regulating gene expression, and can therefore be exploited in a biotechnological setting to "tune" organisms to a certain task.

Das et al. [65] applied QSM isolated from anaerobic sludge to *Chlorella sorokiniana* cultivated in a conventional bubble column reactor. It was reported that bacterial QSMs increased biomass productivity and lipid content of *C. sorokiniana* by 2.25 and 1.28 times, respectively; a finding which could prove important in terms of improving biodiesel production. They also reported higher photosynthetic efficiency and faster settling time after QSM dosing [65]. Another recent study co-cultivated *Chlorella vulgaris* with a bacterium known to produce phytohormones, *Streptomyces rosealbus* [66]. Biosynthesis of IAA was significantly elevated in co-cultivation between these two microorganisms, reaching concentrations of 0.72 μg mL^{-1} (82–140 % higher in reference to the case of monocultures), demonstrated by high performance liquid chromatography (HPLC). Additionally, in response to bacterial IAA production, *C. vulgaris* produced tryptophan to support continued IAA supply, which in turn had positive impact upon microalgal lipid accumulation later in the cultivation period [66]. QSM and various growth-promoting hormones could be beneficial for efficient production of biomass and biodiesel-quality lipids, but supplying these molecules exogenously can be prohibitively expensive. Co-cultivation of different species under conditions that encourage symbiotic interactions is a much more economical pathway to growth promotion via QSM.

However, some signaling molecules can be detrimental to growth and bioproduction. Bacterial lactones and other metabolites can inhibit or inactivate algal gene expression, and otherwise have detrimental impacts on algal growth and reproduction. *Shewanella, Streptomyces*, and *Bacillus* are three bacterial genera known to produce algicidal metabolites [67]. Microalgae can likewise produce compounds (such as acyl homoserine lactones, AHL) which disrupt bacterial quorum sensing; an essential process for biofilm formation. Some species of marine algae are known to excrete fatty acids and halogenated volatile organic compounds which have strong antibacterial activity [67]. Transcriptomics is likely the best tool to ascertain whether certain species can be antagonistic to others in co-culture by providing insights into the functional genes at work within the community.

3.4. Horizontal gene transfer

Many microorganisms are capable of exchanging genetic information with each other, without mating or any form of reproduction. This exchange can occur between different members of all three domains of life (between Eubacteria, Archaea, and Eukarya) [68]. This non-reproductive exchange of genetic information is called horizontal gene transfer. Horizontal gene transfer is best understood between bacterial species, and from bacteria to microalgae. Bacteria offer a wide diversity of metabolically functional genes that more complex microalgae and cyanobacteria incorporate into their genomes. Examples from literature include bacterial genes encoding enzymes for ferritin uptake and for the ornithine urea cycle, transferred from bacteria to diatoms, and helping the diatoms survive iron- and nitrogen-replete conditions, respectively [31]. Transmission in the opposite direction, from eukaryotic microorganisms to prokaryotic, is less well documented. Gene transfer from eukaryotic microalgae to prokaryotic bacteria (including cyanobacteria) likely occurs far less often, though a few examples have been clearly documented [69]. Previously observed only in eukaryotic microalgae, genes for cytoskeletal proteins (actins and tubulins) have been found in the genomes of cyanobacterium *Microcystis aeruginosa* and heterotrophic bacterium *Prosthecobacter*, indicating horizontal gene transfer from eukaryotes [69]. Horizontal gene transfer offers one possible mechanism for the dynamic nature of mixed-species biofilms; environmental pressures may inadvertently select for microorganisms which have acquired useful genes from other consortia partners.

3.5. Enhanced wastewater treatment

Currently, most wastewater treatment schemes do not produce effluents that can be directly used for microalgal cultivation. Most wastewaters require pretreatment prior to use as growth medium for traditional suspended microalgae cultivation in order to maximize photosynthetic efficiency and biomass production. Turbidity of wastewater impede light penetration, constraining photosynthetic efficiency. Toxic components in wastewater could inhibit microalgal growth if not removed prior to use as a cultivation medium. This need for pretreatment hinders the feasibility and scalability for wastewater upgrading to microalgal bioenergy feedstocks on the industrial scale. Additionally, complicated pretreatment steps can increase its water footprint [55]. However, several studies have shown that cultivating mixed-species consortia, especially in biofilms, can reduce the effects of toxicity and turbidity, thereby decreasing the need for costly pretreatments, and ultimately reducing the water footprint of using microalgal bioenergy produced via wastewater treatment [55,70,71]. Diverse biofilms yield versatility in wastewater treatment functionality, as described in the following sections.

3.6. Nutrient and pollutant removal

Natural photosynthetic mats and biofilms have been studied/deployed in natural and artificial ecosystems to reduce nutrient load for decades. The Algal Turf Scrubber (ATS) system was developed in the late 1970′s, patented in 1982, and finally deployed in 1996 with the specific aim of landscape-level nutrient removal and sewage treatment [27]. Nearly-two decades ago, natural cyanobacterial mats were effectively employed to capture nutrient pollution caused by coastal shrimp farming [72]. Filamentous cyanobacteria provided a natural matrix for other, generally pelagic photosynthetic species, including green algae like *Chlorella* and *Dunaliella*, and diatoms such as *Nitzchia* and *Navicula*. This biological matrix also provided a habitat for nitrifying bacteria, such as *Nitrosomonas* and *Nitrobacter,* which converted the ammonium nitrogen species generated by the shrimp to nitrate, which is less toxic and more bioavailable to other members of the consortium. This study reported 95 % and 97 % removal of nitrate and ammonium species, respectively [72].

In the decades following, many studies have demonstrated the value of employing microalgal consortia in nutrient pollution remediation [26,73]. Many more studies have highlighted the benefits of consortia culture in attached cultivation modes, biofilms, or hybrid systems by consistently demonstrating near total removal of nitrogen and phosphorus from wastewater. Hybrid systems, combining both attached and liquid forms of cultivation have shown particular promise. On the laboratory scale, Wicker and Bhatnagar, (2020) achieved up to 92 % and

100 % removal of total nitrogen and phosphate (PO_4^{3-}), respectively, whilst on the pilot scale, Orfanos and Manariotis [74] reported up to 99 % and 93 % removal of nitrate (NO_3^-) and PO_4^{3-}, respectively, from 50 L algal biofilm pond systems. These findings are highly important to consider when designing systems for combined biological waste treatment and biomass production, as they clearly demonstrate how complementary microalgae and bacteria can be to one another in co-culture. Studies elucidating the mechanisms underpinning their nutrient removal efficacy are likewise imperative; for example, microbe-derived dissolved organic nitrogen is a known trigger for harmful cyanobacterial blooms, and the ability to control these biological phenomena is key to process control [75].

Beyond nutrient pollution, microalgal consortia have shown themselves to be uniquely adept at degrading other more toxic pollutants. Testing a conventional *Scenedesmus quadricauda* suspended monoculture Daneshvar et al. [9] examined the ability of living microalgae for biological removal of hexavalent chromium (Cr(VI)) from an aqueous solution. Using a single species cultivated in liquid suspension, the authors found living cells inefficient for Cr(VI) removal, and microalgal biochar to be significantly more effective for Cr(VI) adsorption in comparison. The authors attributed the poor performance of living microalgal cells to the cytotoxic effects of Cr(VI) and its inhibitory impact on cell growth [9]. Conversely, a more recent study investigated an indigenous mixed eukaryotic-prokaryotic microalgal biofilm for nickel removal from synthetic wastewater. Zhou et al. [76] cultivated a mixed photosynthetic consortium in lab-scale rotating algal biofilm (RAB) reactors in 7-day batches with varying concentrations of $NiSO_4{\cdot}6H_2O$. They compared the performance of these microalgal biofilms with conventional suspended cultures in bubble columns inoculated with the same consortium as the RAB systems. The results were striking; while the bubble column removal efficiency averaged around 30 % maximum, the RAB systems exceeded 90 % removal within a range of 100–1000 mg L^{-1} Ni, and still achieved ~ 80 % removal at 5000 mg L^{-1} Ni. The authors report that the biofilms consistently maintained high levels of cell integrity (evidenced by SYTOX Green nucleic acid staining), and physical adsorption of Ni to biofilm EPS in addition to intracellular Ni assimilation. The authors concluded that both the tolerance of microalgal cells to Ni and the high rates of removal from the RAB medium were due to biofilm EPS, which protected cell membranes and adsorbed excess Ni [76].

Mixed-species biofilms are well known to degrade more complex pollutants in natural ecosystems, including phenolic compounds and aromatic hydrocarbons which are abundant in many industrial effluents. This is an important facet of their utility in wastewater management, as these types of compounds are often highly toxic, and may also bioaccumulate and biomagnify when released to the environment. Multiple studies have been conducted exploring the use of mixed consortia and photosynthetic mats to mitigate environmental disasters, such as oil spills. Degradation of aromatic compounds is almost always aerobic in engineered systems; as such, photosynthetic microalgae and cyanobacteria can be very valuable oxygen sources for aerobic bacterial metabolism [77]. A study investigating a microalgal biofilm indigenous in a petrochemical-contaminated stream in Nigeria demonstrated a clear correlation between pollutant degradation and reactive oxygen species (ROS) specifically [57]. Reactive oxygen species (such as peroxides, superoxides, and hydroxyl radicals) are highly reactive oxidants which generate free radicals capable of breaking molecular bonds. By this mechanism, ROS are known to damage biological molecules e.g., DNA, and could likewise degrade petrochemicals by breaking carbon bonds. Although the authors could not identify the species composition of the biofilm, the indigenous biofilm consortium achieved pollutant removal efficiencies, with cadmium, nickel, and lead concentrations reduced by 70, 74, and 71 %, respectively. Additionally, the biofilm improved physicochemical water quality parameters; reducing turbidity, total suspended solids, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) by 71, 66.7, 24, and 33 %, respectively [57].

In another study, two strains of eukaryotic microalgae selected for their tolerance to phenolic compounds (*Scenedesmus obliquus* and *Chlorella vulgaris*) were co-cultured with two bacterial strains known to degrade phenolic compounds (*Raoultella terrigena* and *Pantoea agglomerans*), which were originally isolated from phenol-rich olive washing effluent. Although the bacteria were known to degrade phenol well, the microalgae were considered equally important as producers of oxygen in this system, which is a necessary substrate for the aerobic bacteria to degrade organic material. This artificial consortium was applied to synthetic olive processing wastewater, with phenolic compound concentrations of 50, 100, and 150 mg L^{-1} as the only available carbon source [77]. Remarkably, after a 6-d cultivation period, rates of phenolic compound removal were 60, 50, and 30 % for 50, 100, and 150 mg L^{-1}, respectively. The authors reported maximum consortia growth (which was clearly correlated with nitrogen and phosphorus removal) at the 50 mg L^{-1} concentration, which had eliminated 50 % of phenolic compounds after just 72 h. The authors attributed this efficiency to the process of artificial selection employed to construct the consortium, and the dynamism of mixed species, which establish a natural equilibrium given their environmental conditions [77].

An important study into biological remediation of petroleum industry wastewater demonstrated that the symbiotic associations within microalgal-bacterial consortia contribute significantly to the efficacy of pollutant removal from these effluents. Chavan et al. [78] artificially constructed a consortium of eukaryotic green algae, prokaryotic cyanobacteria, and a heterotrophic bacterium (*Burkholderia cepacia*) known to degrade oil, then investigated the effects of nitrogen and phosphorus ratios on its ability to break down petroleum hydrocarbons. They found that, with a hydraulic retention time of just 21 h, N:P ratios of 19:1, 28.5:1, 38:1, and 47.4:1 yielded 84.6, 97.8, 97.0, and 95.6 % removal of total petroleum hydrocarbons, respectively. These results indicate the tunability and adaptability of such engineered consortia; their performance can be adjusted by manipulating environmental parameters, such as nutrient ratios. These findings have further relevance to the ongoing development of biological methods for oil spill remediation. It has been postulated that prokaryotes such as cyanobacteria play multiple roles in enhancing biological remediation of oil spills; directly, by synergistic interaction and growth promotion of oil-degrading microorganisms, and indirectly, by forming mats which provide a habitat to which these microorganisms can adhere, immobilizing them and preventing tidal turbulence from disrupting their biogeochemical activity [78]. Such studies demonstrate the possibility of developing artificial mat systems seeded with oil-degrading consortia, which can be deployed in pollution zones in the natural environment for on-site biological remediation.

3.7. Carbon capture

Photosynthetic microorganisms are highly adept at capturing carbon in multiple forms. Microalgae (including cyanobacteria) are known to fix CO_2 at a rate 10–50 times faster than land plants, resulting in approximately 1.83 kg CO_2 fixed per 1 kg of biomass produced [1,79,80]. They are capable of efficiently utilizing other forms of inorganic carbon, such as bicarbonate [81], and may also take advantage of organic carbon species during mixotrophic or heterotrophic modes of cultivation. Mixotrophic cultivation of microalgae is optimal, as it maximizes biomass production and total carbon (organic and inorganic) assimilated [82]. Mixotrophy additionally reduces light requirements, which thereby decreases energy costs [83]. However, organic carbon species are much more bioavailable to heterotrophic bacteria. Many common wastewaters and effluents from industries e.g., food processing (dairy) and agricultural digestates (that result from biogas production) contain high amounts of organic carbon, which contributes to chemical and biological oxygen demand [84]. Effective carbon utilization is important in biological waste treatment overall, as it is intricately connected with other biological processes such as growth and nutrient removal. The presence of fixed organic carbon has been shown to

stimulate mixotrophic denitrification in biofilm reactors [85]. Employing mixed species consortia containing heterotrophic bacteria can further enhance organic carbon removal, augmenting the net carbon sequestered by the system via carbon exchange between species. In microalgal-bacterial co-cultures, bacterial respiration of carbon dioxide has been demonstrated to promote microalgal growth and enhance biomass production and quality [86]. Cultivating microalgae in attached systems has shown to increase carbon uptake even further. Guo et al. [87] reported an impressive maximum areal biomass density of 31.44 g m^{-2} and CO_2 removal rate of 65.05 % in a gas-permeable membrane biofilm PBR cultivating *Scenedesmus obliquus*. Relative to their control, this increase in biomass production constituted at least 28 % higher yield, which corresponded with increased carbon capture [87]. Compared with the research cited earlier in this section, this study investigated a eukaryotic microalgal monoculture; the results could potentially be further enhanced by combining a gas-permeable membrane reactor design with aerobic ammonium-oxidizing bacterial co-culture, for example. Mass transfer of CO_2 (and other gases) can be greatly improved by mixed-species biofilm cultivation [88]. Another recent study examined the impact of light intensity upon a *Chlorella* sp. biofilm to sequester carbon, and discovered that light intensity can change the physical structure of photosynthetic biofilms. Wang et al. [89] reported that lower light intensities (20–50 μmol m^{-2} s^{-1}) resulted in more porous biofilm structure and higher photosynthetic potential, which allowed for more efficient CO_2 fixation. Conversely, at higher light intensities (>200 μmol m^{-2} s^{-1}), the biofilm structure became more compact, which inhibited transport of CO_2 and nutrients within the biofilm matrix [89]. Ultimately, designing biological systems to sequester maximal amounts of CO_2 is the best way towards large-scale production of emissions-negative biofuels and bioenergy products.

3.8. Improved biomass production and quality

While traditional suspended microalgal cultivation typically yields a maximum of 0.5 % solid biomass content, attached and/or biofilm cultivation can easily achieve 10–20 % solid biomass yield [90]. This significant improvement in yield can be attributed to several intrinsic characteristics of biofilms. Enhancing mass transfers of nutrient and gas by cultivating mixed-species consortia increases biomass productivity substantially, which has important implications for economic viability of bioreactor and biorefinery systems. A core principle of community ecology is that growth and bioproduction are positively correlated with biodiversity. This correlation can be further explained by resource-use complementarity, reciprocal nutrient exchange, and a reduction in competition between species that occupy different ecological niches [37]. Bulk biomass is naturally increased by co-cultivating multiple diverse species, as they simply occupy space more efficiently (bacterial association with microalgal EPS) [60]. The cell count of microalgae-associated bacteria in the phycosphere can reach a staggering 10^6 bacterial cells mL^{-1} liquid culture, which translates to 100–1000 fold greater than the microalgal cell count [45]. By reducing the physical distance that gases and dissolved nutrients must travel between consortium partners, biofilm or attached cultivation further enhances growth and biomass accumulation.

Attached and/or biofilm cultivation modes can increase the amount of bulk biomass produced by a system, which is imperative for economical production of biofuels and bioenergy. Using ground walnut shells as a self-permeating substrate, Zou et al. [42] designed a novel biofilm photobioreactor for the cultivation of two industrially-relevant microalga, *Scenedesmus obliquus* and *Chlorella vulgaris*. Biomass yields from this system were reported as 97.43 and 70.49 g m^{-2} for *S. obliquus* and *C. vulgaris*, respectively, after 14 d of cultivation. Moreover, using walnuts shells as a substratum had beneficial effects on the lipid content and overall biomass quality. It was postulated that increases in lipid accumulation in microalgal cells could be attributed to increased carbon and nitrogen in the medium from the walnut shell leachate, as well as polyphenols which can increase total lipid yield via antioxidant activity. The maximum lipid yields from *S. obliquus* and *C. vulgaris* cultivated during this study were 34.32 and 28.94 %, respectively; percentages considered to be satisfactory for microalgal biofuel feedstocks [42].

It is well known that the presence of bacteria can enhance microalgal biomass productivity and promote biofilm formation [88]. A study investigating the effects of bacterial co-culture on the industrially-relevant green alga *Chlorella vulgaris* found that the presence of bacteria significantly increased biomass concentration, and also had a positive effect on lipid content [86]. When *C. vulgaris* was cultivated with four strains of bacteria known to promote algal growth (*Flavobacterium, Hypomonas, Rhizobium*, and *Sphingomonas*), Cho et al. [86] reported a biomass concentration of 3.31 g/L, as compared with 1.30 g/L in the control condition (no bacterial counterparts). Additionally, lipid content increased from 22 to 28 % in the co-cultured microalgal cells, with a strong shift towards C16 and C18 fatty acids, which are highly interesting for the purposes of biodiesel production [86].

A recent comparative study between six diverse cyanobacterial species (*Cyanothece* sp., *Nostoc punctiforme*, *Tolypothrix* sp., *Synechocystis* sp., *Synechococcus elongatus*, and *Leptolyngbya* sp.) aimed to evaluate the biofilm-forming capabilities of each strain under nitrate-enriched and nitrate-replete conditions. Bozan et al. [91] also tested co-culturing each cyanobacterial strain with two different "supporter species"; heterotrophic bacteria *Pseudomonas taiwanensis* VLB120 and *Escherichia coli* W3110. The supporter species enhanced biofilm formation by contributing to EPS production. The authors reported that the relatively unknown *Tolypothrix* cyanobacteria performed significantly better than the other five strains in terms of biomass production and biofilm integrity, even under nitrate-deficient conditions (62.6 and 57.5 g/L for nitrate-enriched and nitrate-deficient conditions, respectively) especially when *P. taiwanensis* was the supporter species. Although nitrate concentration had some impact on all strains and supporter species combinations, the key factor for increasing bulk biomass production was strain selection and appropriate co-culturing [91]. Consortium construction is thus a powerful tool in enhancing the quantity and quality of biomass produced by mixed-species biofilm systems.

3.9. Energy-efficient harvesting

A serious bottleneck inhibiting wide-scale utilization of microalgae for production of biofuels and bioenergy is the difficulty and high costs associated with harvesting. Cultivating biofilms or mats is one of the simplest solutions to address this obstacle. Providing that the surface material upon which the organisms are cultured is smooth enough, the biofilm or mat can be removed from the culture medium with minimal mechanical force. In essence, attached or biofilm cultivation combines microalgal growth and flocculation into a single stage. Between biofilm formation and bioflocculation, the principle of biological aggregation is nearly the same, but with greater efficiency and less harvesting time required than separate growth/flocculation stages.

Most reported microalgal biofilms are harvested by simply scraping with a hand tool, although Yu et al. [88] recently developed a novel automated scraper system with the aim of upscaling biofilm cultivation. Harvesting via energy-intensive centrifugation can constitute an average 5–15 % [88], but up to 20–30 % [45] of total production costs associated with microalgal biomass. Alternative to batch harvesting via scraping, continuous harvesting by partially sloughing biomass from surfaces may further reduce energy expenditure, and fully exploit the benefits of continuous cultivation. Biofilms are naturally prone to shear forces during PBR mixing, and this tendency could be intentionally integrated into future biofilm reactor designs.

Connecting a conventional high-rate algal pond (HRAP) to an inclined biofilm reactor, Rodrigues de Assis et al. [92] reported 61 % biomass harvesting of the HRAP/biofilm combination compared with 22 % from the HRAP alone. The addition of the biofilm reactor corresponded with enhanced biodiversity, and increased biomass production

by about 2.6 ×. Finally, combining a biofilm reactor with a conventional HRAP had no negative impacts on wastewater treatment efficacy or the quality of biomass produced. Removal efficiencies of COD, ammonia, and total phosphorus were unaffected by including the biofilm unit, and ratios of protein, carbohydrates, and total lipids were likewise identical between both systems [92]. Such ratios are important for designing subsequent processing pathways; e.g. whether lipid content justifies extraction processes, or if protein content is too high to permit effective anaerobic digestion [93]. HRAP systems are extremely common for wastewater treatment across the globe, and these findings show that retrofitting existing technology with biofilm surfaces can make immense impact on the efficiency of conventional systems.

4. Photobioreactor configurations

The type of photobioreactor implemented can have enormous impacts upon nutrient recovery, carbon capture, biomass production, and the quantities and quality of finished bioproducts. Photobioreactors are classified as either open or closed. Closed systems allow for tighter control over cultivation parameters, and prevent contamination and gas escape much more effectively than open configurations. Closed systems safeguard cultures from outside contamination, and the biomass they produce is often of higher quality; these systems are best suited for targeted production of sensitive biomolecules such as astaxanthin or food-grade omega fatty acid supplements. Open systems, conversely, are advantageous because they are more widely accessible given their simple design, and low construction and operational costs. Open systems may be more prone to contamination, and are unfit for axenic cultivation, but often a better choice when bulk production of low-cost biomass (for upgrading to fuel, rather than food or feed) is the primary objective. Conventional PBRs have been designed largely with the aim of optimizing traditional liquid cultivation in mind. To fully exploit the advantages offered by attached or biofilm cultivation modes, new PBR configurations must be developed and tested. Current PBR configurations, cultivation modes, and advantages vs disadvantages are summarized in Table 2.

Whether designing an open or a closed bioreactor system for attached cultivation, the most important element is choosing a surface for biofilm formation. The material used for biofilm formation is called the carrier. A surface carrier can be made of any material with the ability to adsorb macromolecules or nutrients which are utilized by microalgae or bacteria, and the carrier itself may be stationary or mobile. It is primarily the EPS matrix secreted by biofilm microorganisms, rather than the carrier, which is responsible for immobilizing cells. The EPS additionally adsorbs and stores organic molecules, nutrients, and gases useful to biofilm members [94]. Mature biofilms may separate into aerobic/anaerobic layers (Fig. 3), where, below the oxic/anoxic interface, nutrient cycling in the system may be greatly enhanced by processes such as anammox [94]. Once a surface carrier is selected, a biofilm configuration can be determined based upon the target products or services of the photobioreactor system.

4.1. Open systems

Open PBRs are large pond systems, with either a circular or raceway shape. Circular pond PBRs are stirred with a rotating arm (similar to wastewater treatment ponds), and have been used extensively for the cultivation of *Chlorella* across Asia in recent years [33]. Raceway systems are ponds with an “S” shape, with paddlewheels facilitating water flow. Since their invention in the 1960’s, raceway ponds have largely remained unchanged in design, and have become the most broadly utilized type of open system for industry-scale microalgae cultivation. Biomass productivities between these two types of open systems range 8.5–21 g $(m^2 \cdot d)^{-1}$ for circular ponds and 60–100 mg $(L \cdot d)^{-1}$ for raceway ponds [33].

Open PBRs designed for biofilm cultivation are markedly different in structure as compared with open systems for suspended cultivation. Naumann et al. [95] developed a flat, vertically oriented open

Table 2
Comparison between photobioreactor types and cultivation modes in terms of biomass production and removal of carbon and nutrients.

Cultivation mode	System type	Configuration	Species/consortia type	Biomass yield	Carbon removal (%)	Nitrogen removal (%)	Phosphorus removal (%)	Operational time (d)	Reference
Suspended	Open	High-rate algal pond	Indigenous microalgal-bacterial consortium	0.50 ± 0.03 g/L d^{-1}	85.44	92.68	82.65	28	[92]
	Closed	Glass bottles	*Scenedesmus* sp. and indigenous MWW bacteria	0.2826 g/L d^{-1}	92.3	95.8	98.1	14	[67]
		Jacketed glass tank	*Chlorella sorokiniana* and activated sludge	0.67–0.94 g/L	47	21	54	13	[93]
Hybrid	Open	High-rate algal pond with attached inclined biofilm reactor	Mixed consortium from domestic sewage	93.07 g m^{-2}	59 (COD)	78 (NH_4^+)	16	28	[83]
	Closed	Biofilm membrane (brushes inserted into tank)	*Chlorella vulgaris*	0.072 g/L d^{-1}	–	96 (NH_4^+)	85	15	[87]
		Biofilm airlift (suspended solid carriers)	*Chlorella vulgaris*	0.01593 g/L d^{-1}	–	61.6 ± 7.7	71.3 ± 5.8	37	[94]
Biofilm/ attached	Open	Sloped sanded bed biofilm reactor	Mixed microalgal-bacterial consortium from domestic wastewater treatment	3.8 ± 0.4 g m^{-2} d^{-1}	89 ± 2	92 ± 5	96 ± 2	10	[89]
		Inclined biofilm reactor	*Chlorella* SDEC-18 and indigenous digestate bacteria	5.67 g/L d^{-1}	96.96 (COD)	50.35	94.19	10	[81]
	Closed	Parallel plate reactor	*Scenedesmus obliquus* and filamentous cyanobacteria	26 ± 5 g L^{-1}	75 (COD)	67	96	130	[88]
		Vertical capillary-driven photobioreactor	*Scenedesmus* sp. LX1	121.5 g m^{-2}	–	96	80	12	[82]

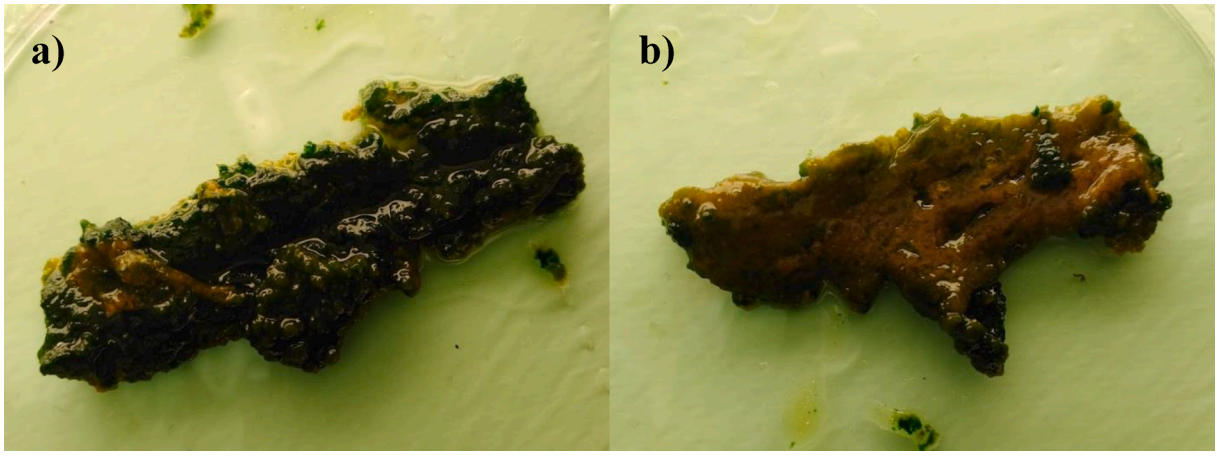

Fig. 3. A mixed-species bilayer biofilm; a) the top-facing, photosynthetic layer, containing filamentous cyanobacteria and immobilized microalgae, b) the bottom-facing layer, comprised mainly of heterotrophic bacteria, which had been directly attached to a plastic scaffold material prior to excision.

photobioreactor for solid-state cultivation of typically pelagic microalgae important in the aquaculture industry (*Isochrysis* sp., *Tetraselmis suecica*, *Phaeodactylum tricornutum, Nannochloropsis* sp.). Although not strictly cultivated as a biofilm, these microalgae were immobilized by "self-adhesion" to a printer paper substrate layer, which was laid on top of a glass fiber source layer, through which the liquid culture medium could flow to the immobilized cells. This dual-layer configuration separated microalgal cells from the culture medium, which was easily harvested by hand with a plastic scraper. This novel system yielded concentrated microalgal paste (72–84 % water content) with dry weight equivalents of 10–15 g m^{-2} after a cultivation period of 14–25 d [95]. Another more recent study reported the development of an inclined tank system which evenly distributed the culture medium (anaerobically-digested kitchen waste effluent diluted with seawater) over substrate troughs, which kept biofilms exposed to ambient air. This system was particularly efficient at removing phosphorus and degrading organic material present in the wastewater used, likely due to excess oxygen available to indigenous bacteria species from the anaerobic digestion process [88].

4.2. Closed systems

Closed PBR systems are much more diverse in configuration than open systems. Closed PBR designs include flat plate (or panel), airlift or bubble column, horizontal tube (or spiral), and stirred tanks [33]. Improvement of closed systems is ongoing; a recent study conducted by Khoobkar et al. [96] arranged four flat panel PBRs in a pyramidal configuration to optimize surface area and light penetration (from within the pyramid and outside of it). The addition of an internal light source to this configuration had a significantly positive impact upon photosynthetic efficiency and biomass production, with an increase of up to 4.2 mg L^{-1} d^{-1} with respect to the control (only external lighting). Such advances, however, can be greatly enhanced by incorporating biofilm cultivation.

Flat plate bioreactors are perhaps the most easily modified closed PBR systems to accommodate microalgal biofilms. These systems are hybridized; they allow for suspended and attached cultivation simultaneously. Gao et al. [97] inserted flexible fiber bundles into a plexiglass cultivation tank with the aim of increasing biomass production and harvesting efficiency; after harvesting the total biomass produced, they showed that 72.4 % of the harvested biomass had been immobilized in the fiber bundles. A laboratory-scale study aimed to develop a dual-phase rooftop system with a liquid chemical adsorption phase followed by a biofilm stage for nutrient recovery from domestic wastewater [98]. This "decentralized" system (i.e. completely operable independent of external wastewater treatment services) reported removal rates of COD and phosphorus which met European Union regulations, although nitrogen removal fell just short of the legal limit for discharge. The biofilms, cultivated in a parallel plate system, produced an impressive 26 ± 5 g dry biomass per liter, and showed cooperative consortium development over 130 d of operation. Although the experiment began with *Scenedesmus obliquus* as the dominant biofilm species, by the end of the experiment, the biofilm had matured with filamentous cyanobacteria (*Phormidium* sp. and *Oscillatoria* sp. identified by scanning electron microscopy), unidentified non-photosynthetic bacteria attached to cyanobacterial filaments, and *S. obliquus* alongside diatoms embedded in the biofilm [98]. These findings suggest that despite nitrogen recovery initially falling short of EU discharge standards, intentionally increasing biodiversity by including nitrifying bacteria in consortia with other microalgal species could greatly improve the efficacy of such systems.

Another study explored the benefits of mixotrophy in microalgal biofilms and compared four different surface materials with variable roughness inserted into cultivation tanks (stainless steel, polypropylene, acrylic, and polycarbonate) [83]. Biofilm cultivation under mixotrophic conditions (both organic and inorganic carbon species available simultaneously) yielded 2–3 times more biomass, 2–10 times more lipids, and 40–60 % lower ash content, all of which are highly significant for effective and economical biofuel production [83]. Mixotrophy is a valuable tool to maximize carbon uptake and biomass productions whilst reducing lighting requirements and related operational costs, and should be considered when designing photobioreactors of any type.

4.3. Scalability

Studies such as those conducted by Naumann et al. [95] and Zamalloa et al. [98] demonstrate that immobilized, attached, and biofilm cultivation modes for microalgae can be scaled up effectively without accruing prohibitive expense. In the case of the twin-layer solid state PBR [95], the system was operated under greenhouse conditions using natural sunlight in Köln, Germany, and utilized commercial printer paper as a substrate. Additionally, and in contrast with the conventional open photobioreactor systems, an energy requirement of approximately 1 kWh per 1 kg of dry biomass represents a significant reduction in energetic expense; estimated by the authors to reduce energy input by a factor of 10 [95]. However, when comparing a closed tubular PBR and an open biofilm system, Posadas et al. [99] found that the open biofilm PBR was superior to the tubular system in removing nutrients from domestic wastewater. After a hydraulic retention time of 10 d, they reported approximately 100 % inorganic carbon removal, as well as removal efficiencies of 89 ± 2, 92 ± 5, and 96 ± 2 % for organic carbon, nitrogen, and phosphorus, respectively, in the open biofilm system [99]. The pilot-scale HRAP combined with an inclined biofilm

plate reported by Rodrigues de Assis et al. [92] (discussed in Section 3.4) offers more evidence that biofilm cultivation is highly compatible with open PBR configurations, and can help to address the most persistent bottlenecks associated with scalability of microalgal cultivation, namely biomass production and harvesting efficiency. Findings such as these have important implications for scaling up biofilm cultivation, as open systems such as ponds and open bed biofilms reactors are generally much more affordable and accessible technology in most parts of the world, applicable even at Nordic latitudes [100].

5. Upgrading biomass to biofuels and bioenergy products

Once sufficient biomass has been generated by biofilm cultivation, it typically will not require significant pre-processing after harvesting (e.g. dewatering via flocculation, centrifugation, or other methods), although the efficiency of most upgrading pathways is greatly enhanced by some form of preliminary cell disruption to liberate cellular components. In order to maximize energetic efficiency of the total bioenergy/biofuel production system, 1) upgrading methods compatible with wet biomass should be selected, and 2) a zero-waste biorefinery scheme should be adopted. Most waste streams generated by current state-of-the-art upgrading pathways can be further valorized to other value-added products (VAPs) to increase the total value extracted from the biomass (Table 3). Chemical and biochemical upgrading pathways from biofilm biomass to various biofuels and bioenergy products are illustrated in Fig. 4.

5.1. Chemical and thermochemical pathways

One of the most mature chemical upgrading pathways for biofuel production is transesterification of extracted lipids. Conventional transesterification requires high energy input for drying biomass completely, as well as expensive and toxic reagents for lipid extraction and catalysis. However, numerous studies in the past decade have focused on improving transesterification from each of these perspectives. The wet lipid extraction process (WLEP) was developed by Sathish and Sims [101] to bypass the drying stage entirely, and showed great promise for integration with other biorefinery processes. WLEP allows for direct (or *in situ*) transesterification by simultaneous extraction of lipids from dewatered (up to 20 % water content) biomass and reaction with excess methanol [102]. Apart from high-purity microalgal lipids, side streams of WLEP include lipid-extracted residual biomass with a favorable C/N ratio (54.6:1) for further biological upgrading, an aqueous phase containing valuable nitrogen, phosphorus, and carbon species, and a solid precipitate containing 70 % proteins [103]. Each of these side streams can be utilized as products such as biofertilizer, or valorized as rich feedstocks for other upgrading pathways, such as anaerobic digestion.

Beyond transesterification pathways to produce conventional biodiesel, thermochemical conversion methods (developed originally for petroleum refining) have been applied to microalgal biomass to achieve liquid fuels with different properties for more targeted applications, such as aviation fuel [104]. Thermochemical techniques, such as pyrolysis, liquefaction, and gasification require high temperatures and pressures which are prohibitively expensive at present. However, if the cost of producing high-quality biomass can be significantly reduced by attached or biofilm cultivation, the costs of thermochemical conversion could be mitigated by the sale energy-dense end products. An early study [105] comparing the bio-crude oil derived from liquefaction and pyrolysis of wet microalgal biomass (~80 % water content) reported similar properties for raw *Arthospira*, and raw *Scenedesmus*, and lipid-extracted *Scenedesmus* in terms of heating values, heteroatom content, and functionality, but chemical analyses revealed some differences between these two thermochemical processing techniques. Hydrothermal liquefaction had a better energy balance for biomass containing approximately 80 % moisture, although slow pyrolysis produced oils with lower molecular weights and boiling points, and higher percentages of cyclic oxygenates. Both conversion methods, however, produced bio-oils with energy densities between 35 and 37 MJ/kg [105]. More recently, Van Doren et al. [106] evaluated the potential for energy recovery from hydrothermal liquefaction of waste biomass coupled with either anaerobic digestion or catalytic hydrothermal gasification. After modelling four different scenarios and conducting a technoeconomic analysis, they found that a large percentage of the energy content of microalgal biomass remains in the aqueous phase as soluble organic carbon. This carbon-rich aqueous phase is ideal for subsequent upgrading via anaerobic digestion to biogas, which can be used directly for heating or thermoelectric power generation [106].

Hydrothermal liquefaction is ideally applied to wet biomass with a maximum of 80 % moisture content [105], which corresponds exactly with the moisture content of biofilm biomass (without the need for dewatering or drying) (Section 3.3). Considering the favorable energy balance of thermochemical conversion techniques, these methods should be developed further to reduce energy costs and improve quality and yield of target biofuels, and integrated with other biorefinery processes (e.g. anaerobic digestion) to minimize waste and maximize economic returns.

5.2. Biochemical pathways

Biochemical upgrading pathways involve other types of cultivated microorganisms such as heterotrophic *Clostridium* bacteria or *Saccharomyces* yeast, used extensively across various industries for anaerobic digestion to biogas [93] and fermentation to alcohols [107], respectively. *Clostridia* sp. and some other types of bacteria (e.g., *Enterococcus*) are also capable of acetone/butanol/ethanol (ABE) fermentation [108], which can be integrated with biological hydrogen gas production [109]. Contrasted with other abundant and low-cost bio-ethanol feedstocks,

Table 3
Biofuels and bioenergy products that can be derived from microalgal biomass, including target bioprocesses during cultivation and possible co-products obtainable via biorefinery pathways.

State	Fuel	Microalgal bioprocesses	Upstream processes	Co-products	Applications	References
Gas	Methane	Carbohydrate accumulation	Anaerobic digestion of microalgal biomass	CO_2, nutrient-rich sludge (usable as fertilizer)	Direct heating, thermoelectric power generation	[30]
	Hydrogen	Photobiological production	Physical gas separation/ purification	Microalgal biomass	Combustion engines for heating or power generation, hydrogen fuel cells	[106,107]
Liquid	Biodiesel	Lipid accumulation	Transesterification	Residual (lipid-extracted) biomass	Gasoline/diesel fuel replacement	[93,108]
	Bio-crude oil		Liquefaction, pyrolysis	Nutrient-rich aqueous and gaseous side streams	Transportation fuel, usable in various types of internal combustion engines	[96,97]
	Bio-jet fuel		Transesterification followed by hydro-processing	Residual biomass, nutrient-rich, aqueous phase, CO_2	Aviation fuel	[95]
	Ethanol	Carbohydrate accumulation	Fermentation of pre-treated or lipid-extracted biomass	CO_2, nutrient-rich sludge	Liquid fuel additive	[98,99]
	Butanol			Acetone (ABE fermentation)		[99,100]

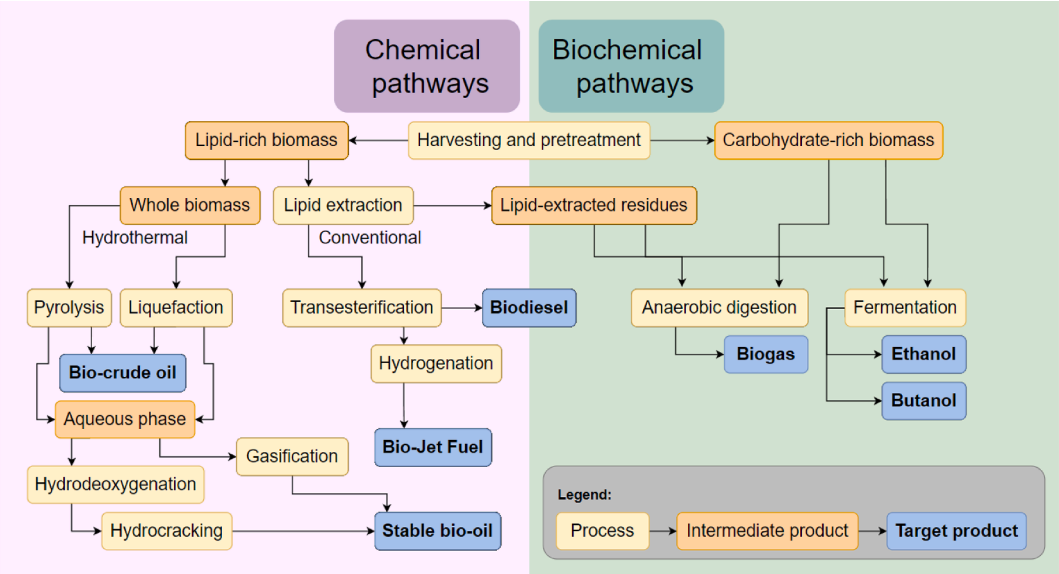

Fig. 4. Chemical and biochemical pathways available for upgrading biofilm biomass into fungible biofuels and bioenergy products.

such as agriculture waste, microalgal biomass does not contain complex organic molecules like lignin, which require more complex pre-treatments prior to fermentation [107]. Recent advances in chemo-enzymatic hydrolysis of microalgal biomass have resulted in some of the highest ethanol yields from microalgae in the past several years; Constantino et al. [110] reported 82–91 % ethanol yields (of theoretical values) after hydrothermal acid pretreatment followed by a reverse-order two-step enzymatic hydrolysis (amyloglucosidase followed by α-amylase, the reverse order of conventional enzymatic hydrolysis of starch) [110]. Using thermostable enzymes, Shokrkar et al. [107] achieved up to 92 % theoretical yield from wet mixed-species microalgal biomass without applying extreme temperatures or strong acids. Fermentation is perhaps the most mature and energy-efficient biotechnology available, and its products are broadly applicable in the energy sector as well as in various other industries.

In many cases, biomass obtained from biofilm cultivation will contain relatively high amounts of carbohydrates, due to high polysaccharide content of the EPS matrix, and the general tendency of colonial and filamentous microalgae/cyanobacteria towards carbohydrate accumulation rather than lipid storage. However, care must be taken to characterize biofilm compositions such that appropriate downstream processes can be selected. For example, a biofilm comprised mainly of carbohydrates with very low protein and lipid content would likely be most effectively valorized to bioenergy products by forgoing any extraction or fractionation processes and subjecting the whole biomass directly to enzymatic hydrolysis, followed by fermentation or anaerobic digestion. Conversely, biomass with substantial lipid content or protein levels is better suited for fractionation, as lipids, proteins, and carbohydrates each require different processing pathways to generate high-quality biofuels, bioenergy products, and other saleable VAPs.

6. Future perspectives

Given the recent advances highlighted in this review, attached and biofilm cultivation of mixed-species consortia should be pursued as an affordable, accessible biotechnology to address multiple industrial challenges related to the widespread production of microalgal biofuels and bioenergy. These challenges include pollution mitigation, carbon capture, and bioproduction of numerous high-value co-products. By reducing operational costs of microalgal biorefinery systems, especially at the harvesting stage, attached and biofilm modes of cultivation can make microalgal biofuels and bioenergy products economically feasible, even in the least-developed economies.

More research is required to develop efficient, scalable photobioreactor systems that facilitate attached cultivation and low-energy biomass harvesting, although much of the physical basis for these systems is already in place. Perhaps the most straightforward way to integrate mixed-species photosynthetic biofilm cultivation into current wastewater infrastructure is to adapt tertiary wastewater treatment ponds (Fig. 5). A high surface area transparent material overlaying the ponds could provide the biofilm substrate; transparent to allow light to penetrate from above, modular to allow for easy harvesting. Most wastewater treatment plants worldwide have indigenous photosynthetic consortia growing in the system already. The most difficult part of taking advantage of these consortia is isolating them, sequencing their genomes and transcriptomes, and adapting them to grow as a specialized biofilm consortium. This last phase may require consortium construction; e.g. incorporation of other microorganisms (e.g. filamentous cyanobacteria species to form the base of the biofilm), or removal of harmful species (e.g. bacteria which produce algicidal metabolites). In practical terms, this process could be undertaken without the use of sophisticated genomic or transcriptomic analyses, and rather fine-tuned using chemical analyses of wastewater treatment over time by an indigenous consortium. The replicability of mixed-species biofilms remains a challenge; it is nearly to "clone" a diverse, dynamic consortium. However, considering purely pragmatic wastewater treatment and bioproduction/bioenergy goals, the species makeup is relatively unimportant, so long as the function of the community suits the industrial needs.

Future studies should investigate the combination of mixed-species consortia in biofilms or other attached cultivation modes, as most current work focuses either on attached cultivation or algal-bacterial symbiosis, and not both simultaneously, especially in an industrial context. Additional work is needed on the long-term feasibility of mixed-species biofilms, as current literature reports experimental periods of weeks, rather than months or years. Given the dynamic nature of mixed consortia, long-term studies on control processes for community structure and function would greatly improve the industrial lifespan and viability of systems. Biological mechanisms of growth promotion and other symbiotic interactions must yet be elucidated to maximize the efficacy and net energetic yields of biofuels and bioenergy produced by mixed-species biofilm systems in the future. Biotechnological systems must mimic nature going forward, rather than trying to pigeonhole species of interest into our current infrastructure. At the same time, research should aim to intelligently engineer artificial consortia to suit

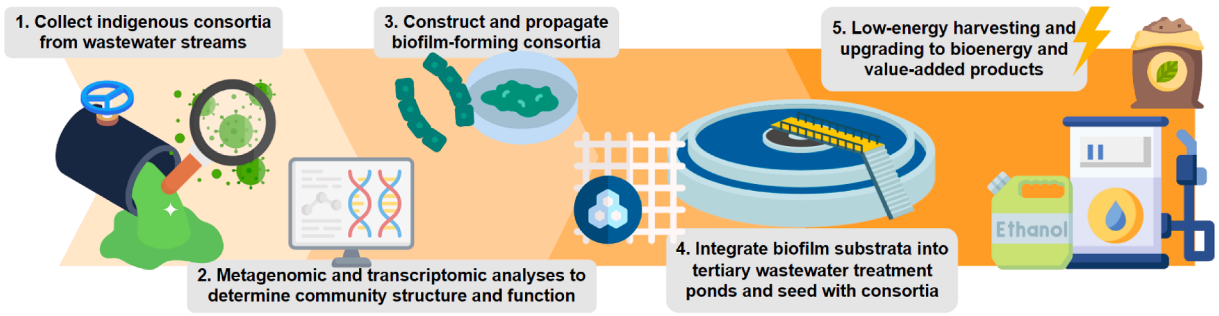

Fig. 5. A proposed operational strategy model for integrating photosynthetic mixed-species biofilms for targeted wastewater treatment and economical production of biomass for valorization to bioenergy and bioproducts.

these systems in order to maximize their potential to meet ever-growing biofuel and bioenergy demands and emissions reduction targets.

7. Conclusions

Considering the findings of this review, a hybrid approach is recommended, using mixed-species consortia with attached or biofilm cultivation for effective and economical biomass production. In doing so, the resilience and versatility of mixed species consortia are combined with the efficacy of biofilm biomass production and low-energy harvesting, synergistically addressing multiple current issues in microalgal biofuel and bioenergy production. The most robust and sophisticated biological systems are those developed by nature, and mixed-species biofilms have persisted throughout earth's natural history. Industrially and economically viable microalgal biofuels and bioenergy can flourish by taking a few key lessons from evolutionary history.

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

No data was used for the research described in the article.

Acknowledgements

Icons in the graphical abstract have been sourced from www.flaticon.com, and the authors wish to thank their creative team. The first author Rebecca J. Wicker gratefully acknowledges financial support from Tekniikan Edistämissäätiö (Grant number: 8883). All authors sincerely express their appreciation and thanks to the reviewers whose critical commentary has significantly improved the quality of this publication.

References

[1] J.A.V. Costa, G.A. Linde, D.I.P. Atala, G.M. Mibielli, R.T. KrÜger, Modelling of growth conditions for cyanobacterium Spirulina platensis in microcosms, World Journal of Microbiology and Biotechnology (2000), https://doi.org/10.1023/A:1008992826344.
[2] X. Miao, Q. Wu, Biodiesel production from heterotrophic microalgal oil, Bioresource Technology 97 (6) (2006) 841–846.
[3] Y.-J. Lei, Y.u. Tian, J. Zhang, L.i. Sun, X.-W. Kong, W. Zuo, L.-C. Kong, Microalgae cultivation and nutrients removal from sewage sludge after ozonizing in algal-bacteria system, Ecotoxicology and Environmental Safety 165 (2018) 107–114.
[4] M.-K. Ji, H.-S. Yun, B.S. Hwang, A.N. Kabra, B.-H. Jeon, J. Choi, Mixotrophic cultivation of Nephroselmis sp. using industrial wastewater for enhanced microalgal biomass production, Ecological Engineering 95 (2016) 527–533.
[5] E. Daneshvar, M.J. Zarrinmehr, E. Koutra, M. Kornaros, O. Farhadian, A. Bhatnagar, Sequential cultivation of microalgae in raw and recycled dairy wastewater: Microalgal growth, wastewater treatment and biochemical composition, Bioresource Technology 273 (2019) 556–564.
[6] R. Wicker, A. Bhatnagar, Application of Nordic microalgal-bacterial consortia for nutrient removal from wastewater, Chemical Engineering Journal 398 (2020) 125567.
[7] M. Jämsä, F. Lynch, A. Santana-Sánchez, P. Laaksonen, G. Zaitsev, A. Solovchenko, Y. Allahverdiyeva, Nutrient removal and biodiesel feedstock potential of green alga UHCC00027 grown in municipal wastewater under Nordic conditions, Algal Research 26 (2017) 65–73.
[8] A. Aslam, S.R. Thomas-Hall, T. Mughal, Q.-u. Zaman, N. Ehsan, S. Javied, P. M. Schenk, Heavy metal bioremediation of coal-fired flue gas using microalgae under different CO 2 concentrations, Journal of Environmental Management 241 (2019) 243–250.
[9] E. Daneshvar, M.J. Zarrinmehr, M. Kousha, A.M. Hashtjin, G.D. Saratale, A. Maiti, M. Vithanage, A. Bhatnagar, Hexavalent chromium removal from water by microalgal-based materials: Adsorption, desorption and recovery studies, Bioresource Technology 293 (2019) 122064.
[10] E. Daneshvar, M.J. Zarrinmehr, A.M. Hashtjin, O. Farhadian, A. Bhatnagar, Versatile applications of freshwater and marine water microalgae in dairy wastewater treatment, lipid extraction and tetracycline biosorption, Bioresource Technology 268 (2018) 523–530.
[11] H.N.P. Vo, H.H. Ngo, W. Guo, K.H. Nguyen, S.W. Chang, D.D. Nguyen, Y. Liu, Y. i. Liu, A.n. Ding, X.T. Bui, Micropollutants cometabolism of microalgae for wastewater remediation: Effect of carbon sources to cometabolism and degradation products, Water Research 183 (2020) 115974.
[12] A.K. Koyande, K.W. Chew, K. Rambabu, Y. Tao, D.-T. Chu, P.-L. Show, Microalgae: A potential alternative to health supplementation for humans, Food Science and Human Wellness 8 (1) (2019) 16–24.
[13] M.A. Borowitzka, Commercial production of microalgae: ponds, tanks, tubes and fermenters. J. Biotechnol., 70, 313– 321, Journal of Biotechnology. (1999).
[14] Y. Najm, S. Jeong, TorOve Leiknes, Nutrient utilization and oxygen production by Chlorella vulgaris in a hybrid membrane bioreactor and algal membrane photobioreactor system, Bioresource Technology 237 (2017) 64–71.
[15] H. Sati, M. Mitra, S. Mishra, P. Baredar, Microalgal lipid extraction strategies for biodiesel production: A review, Algal Research 38 (2019) 101413.
[16] A.S. Japar, M.S. Takriff, N.H.M. Yasin, Harvesting microalgal biomass and lipid extraction for potential biofuel production: A review, Journal of Environmental Chemical Engineering 5 (1) (2017) 555–563.
[17] C.-H. Sun, Q. Fu, Q. Liao, A.o. Xia, Y. Huang, X. Zhu, A. Reungsang, H.-X. Chang, Life-cycle assessment of biofuel production from microalgae via various bioenergy conversion systems, Energy 171 (2019) 1033–1045.
[18] J.W. Richardson, M.D. Johnson, R. Lacey, J. Oyler, S. Capareda, Harvesting and extraction technology contributions to algae biofuels economic viability, Algal Research 5 (2014) 70–78.
[19] D.E. Canfield, Oxygen: A Four Billion Year History, 2015. https://doi.org/10.1515/9781400849888.
[20] W. Bell, R. Mitchell, Chemotactic and growth responses of marine bacteria to algal extracellular products, The Biological Bulletin 143 (2) (1972) 265–277.
[21] V. Scognamiglio, M.T. Giardi, D. Zappi, E. Touloupakis, A. Antonacci, Photoautotrophs–Bacteria Co-Cultures: Advances, Challenges and Applications, Materials 2021, Vol. 14, Page 3027. 14 (2021) 3027. https://doi.org/10.3390/MA14113027.
[22] G. Kwon, H. Kim, C. Song, D. Jahng, Co-culture of microalgae and enriched nitrifying bacteria for energy-efficient nitrification, Biochemical Engineering Journal 152 (2019) 107385.
[23] V.Z. dos Santos, K.R. Vieira, P.P. Nass, L.Q. Zepka, E. Jacob-Lopes, Application of Microalgae Consortia/Cocultures in Wastewater Treatment (2021) 131–154, https://doi.org/10.1007/978-981-16-0518-5_5.
[24] M. Wang, R. Keeley, N. Zalivina, T. Halfhide, K. Scott, Q. Zhang, P. van der Steen, S.J. Ergas, Advances in algal-prokaryotic wastewater treatment: A review of nitrogen transformations, reactor configurations and molecular tools, Journal of Environmental Management 217 (2018) 845–857.
[25] G. Flores-Salgado, F. Thalasso, G. Buitrón, M. Vital-Jácome, G. Quijano, Kinetic characterization of microalgal-bacterial systems: contributions of microalgae and heterotrophic bacteria to the oxygen balance in wastewater treatment, Biochemical Engineering Journal 165 (2021) 107819.

[26] B. Ji, M. Zhang, J. Gu, Y. Ma, Y. Liu, A self-sustaining synergetic microalgal-bacterial granular sludge process towards energy-efficient and environmentally sustainable municipal wastewater treatment, Water Res 179 (2020), 115884, https://doi.org/10.1016/j.watres.2020.115884.

[27] W.H. Adey, P.C. Kangas, W. Mulbry, Algal Turf Scrubbing: Cleaning Surface Waters with Solar Energy while Producing a Biofuel, BioScience 61 (6) (2011) 434–441.

[28] P. Kangas, W. Mulbry, P. Klavon, H.D. Laughinghouse, W. Adey, High diversity within the periphyton community of an algal turf scrubber on the Susquehanna River, Ecological Engineering 108 (2017) 564–572.

[29] Z. Zhao, K. Muylaert, A. Szymczyk, I.F.J.J. Vankelecom, Enhanced microalgal biofilm formation and facilitated microalgae harvesting using a novel pH-responsive, crosslinked patterned and vibrating membrane, Chemical Engineering Journal 410 (2020), 127390, https://doi.org/10.1016/j.cej.2020.127390.

[30] F. Witarsa, A. Yarberry, P. May, P. Kangas, S. Lansing, Complementing energy production with nutrient management: Anaerobic digestion system for algal turf scrubber biomass, Ecological Engineering 143 (2020) 105618.

[31] B. Zhang, W. Li, Y. Guo, Z. Zhang, W. Shi, F. Cui, P.N.L. Lens, J.H. Tay, Microalgal-bacterial consortia: From interspecies interactions to biotechnological applications, Renewable and Sustainable Energy Reviews 118 (2020) 109563.

[32] B. Podola, T. Li, M. Melkonian, Porous Substrate Bioreactors: A Paradigm Shift in Microalgal Biotechnology? Trends in Biotechnology 35 (2) (2017) 121–132.

[33] H. Ting, L. Haifeng, M. Shanshan, Y. Zhang, L. Zhidan, D. Na, Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: A review, Chinese Society of, Agricultural Engineering (2017).

[34] A.L. Gonçalves, J.C.M. Pires, M. Simões, A review on the use of microalgal consortia for wastewater treatment, Algal Research 24 (2017) 403–415.

[35] J. Wang, W. Liu, T. Liu, Biofilm based attached cultivation technology for microalgal biorefineries—A review, Bioresource Technology 244 (2017) 1245–1253, https://doi.org/10.1016/J.BIORTECH.2017.05.136.

[36] J. Liu, Y. Wu, C. Wu, K. Muylaert, W. Vyverman, H.Q. Yu, R. Muñoz, B. Rittmann, Advanced nutrient removal from surface water by a consortium of attached microalgae and bacteria: A review, Bioresource Technology 241 (2017) 1127–1137, https://doi.org/10.1016/J.BIORTECH.2017.06.054.

[37] E. Kazamia, A.S. Riseley, C.J. Howe, A.G. Smith, An Engineered Community Approach for Industrial Cultivation of Microalgae, Industrial Biotechnology 10 (3) (2014) 184–190.

[38] N. Shigesada, A. Okubo, Analysis of the self-shading effect on algal vertical distribution in natural waters, J Math Biology 12 (3) (1981) 311–326.

[39] E. Sforza, M. Pastore, S.M. Franke, E. Barbera, Modeling the oxygen inhibition in microalgae: An experimental approach based on photorespirometry, New Biotechnology 59 (2020) 26–32, https://doi.org/10.1016/J.NBT.2020.06.003.

[40] F. Iasimone, J. Seira, A. Panico, V. de Felice, F. Pirozzi, J.P. Steyer, Insights into bioflocculation of filamentous cyanobacteria, microalgae and their mixture for a low-cost biomass harvesting system, Environmental Research 199 (2021), 111359, https://doi.org/10.1016/J.ENVRES.2021.111359.

[41] A. Mantzorou, F. Ververidis, Microalgal biofilms: A further step over current microalgal cultivation techniques, Science of The Total Environment 651 (2019) 3187–3201, https://doi.org/10.1016/J.SCITOTENV.2018.09.355.

[42] X. Zou, K. Xu, W. Chang, Y. Qu, Y. Li, A novel microalgal biofilm reactor using walnut shell as substratum for microalgae biofilm cultivation and lipid accumulation, Renewable Energy 175 (2021) 676–685, https://doi.org/10.1016/J.RENENE.2021.04.122.

[43] S. Meng, X. Meng, W. Fan, D. Liang, L. Wang, W. Zhang, Y. Liu, The role of transparent exopolymer particles (TEP) in membrane fouling: A critical review, Water Research 181 (2020), 115930, https://doi.org/10.1016/J.WATRES.2020.115930.

[44] W. Chen, J. Mo, X. Du, Z. Zhang, W. Zhang, Biomimetic dynamic membrane for aquatic dye removal, Water Research 151 (2019) 243–251, https://doi.org/10.1016/J.WATRES.2018.11.078.

[45] H. Wang, R.T. Hill, T. Zheng, X. Hu, B. Wang, Effects of bacterial communities on biofuel-producing microalgae: Stimulation, inhibition and harvesting, Critical Reviews in Biotechnology 36 (2) (2016) 341–352.

[46] C. Banerjee, S. Ghosh, G. Sen, S. Mishra, P. Shukla, R. Bandopadhyay, Study of algal biomass harvesting through cationic cassia gum, a natural plant based biopolymer, Bioresource Technology 151 (2014) 6–11.

[47] W. Zhou, M. Min, B. Hu, X. Ma, Y. Liu, Q. Wang, J. Shi, P. Chen, R. Ruan, Filamentous fungi assisted bio-flocculation: A novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells, Separation and Purification Technology 107 (2013) 158–165.

[48] C.H.T. Vu, H.-G. Lee, Y.K. Chang, H.-M. Oh, Axenic cultures for microalgal biotechnology: Establishment, assessment, maintenance, and applications, Biotechnology Advances 36 (2) (2018) 380–396.

[49] J.J. Cole, Interactions between bacteria and algae in aquatic ecosystems, Annual Review of Ecology and Systematics 13 (1) (1982) 291–314.

[50] S. Sabater, X. Timoner, C. Borrego, V. Acuña, Stream Biofilm Responses to Flow Intermittency: From Cells to Ecosystems, Frontiers in Environmental Science (2016) 14, https://doi.org/10.3389/FENVS.2016.00014.

[51] S.R. Subashchandrabose, B. Ramakrishnan, M. Megharaj, K. Venkateswarlu, R. Naidu, Consortia of cyanobacteria/microalgae and bacteria: Biotechnological potential, Biotechnology Advances 29 (6) (2011) 896–907.

[52] E. Kazamia, D.C. Aldridge, A.G. Smith, Synthetic ecology - A way forward for sustainable algal biofuel production? Journal of Biotechnology 162 (1) (2012) 163–169.

[53] G.A. Lutzu, Interactions of microalgae and other microorganisms for enhanced production of high-value compounds, Frontiers in Bioscience - Landmark 23 (8) (2018) 1487–1504.

[54] M. Takahashi, S. Kato, H. Shima, E. Sarai, T. Ichioka, S. Hatyakawa, H. Miyajiri, Technology for recovering phosphorus from incinerated wastewater treatment sludge, Chemosphere 44 (1) (2001) 23–29.

[55] E.S.J. Thoré, F. Schoeters, A. de Cuyper, R. Vleugels, I. Noyens, P. Bleyen, S. van Miert, Waste Is the New Wealth – Recovering Resources From Poultry Wastewater for Multifunctional Microalgae Feedstock, Frontiers in Environmental Science (2021) 253, https://doi.org/10.3389/FENVS.2021.679917.

[56] P.K. Akao, B. Singh, P. Kaur, A. Sor, A. Avni, A. Dhir, S. Verma, S. Kapoor, U. G. Phutela, S. Satpute, S. Sharma, D. Avisar, K.S. Sandha, H. Mamane, Coupled microalgal–bacterial biofilm for enhanced wastewater treatment without energy investment, Journal of Water Process Engineering 41 (2021), 102029, https://doi.org/10.1016/J.JWPE.2021.102029.

[57] A.Y. Ugya, F.O. Ajibade, X. Hua, The efficiency of microalgae biofilm in the phycoremediation of water from River Kaduna, Journal of Environmental Management 295 (2021), 113109, https://doi.org/10.1016/J.JENVMAN.2021.113109.

[58] T. Watari, Y. Fukushima, T. Miwa, Y. Hirakata, S. Kawakami, Y. Nakamura, M. Hatamoto, T. Yamaguchi, Development of a photo-baffled reactor for microalgae-nitrifying bacteria consortia: Achieving long-term, stable partial nitrification, Journal of Environmental Chemical Engineering 9 (5) (2021) 106082.

[59] J. Fuentes, I. Garbayo, M. Cuaresma, Z. Montero, M. González-del-Valle, C. Vílchez, Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds, Marine Drugs 14 (5) (2016) 100.

[60] S. Yao, S. Lyu, Y. An, J. Lu, C. Gjermansen, A. Schramm, Microalgae–bacteria symbiosis in microalgal growth and biofuel production: a review, Journal of Applied Microbiology 126 (2019) 359–368, https://doi.org/10.1111/JAM.14095.

[61] C.C. Tang, X. Zhang, Z.W. He, Y. Tian, X.C. Wang, Role of extracellular polymeric substances on nutrients storage and transfer in algal-bacteria symbiosis sludge system treating wastewater, Bioresource Technology 331 (2021), 125010, https://doi.org/10.1016/J.BIORTECH.2021.125010.

[62] D.E. Canfield, A.N. Glazer, P.G. Falkowski, The Evolution and Future of Earth's Nitrogen Cycle, Science 330 (2010) (1979) 192–196, https://doi.org/10.1126/SCIENCE.1186120.

[63] Y. Zheng, Y. Huang, Q. Liao, Q. Fu, A. Xia, X. Zhu, Impact of the accumulation and adhesion of released oxygen during Scenedesmus obliquus photosynthesis on biofilm formation and growth, Bioresource Technology 244 (2017) 198–205, https://doi.org/10.1016/J.BIORTECH.2017.07.145.

[64] J. Zhou, Y. Lyu, M.L. Richlen, D.M. Anderson, Z. Cai, Quorum Sensing Is a Language of Chemical Signals and Plays an Ecological Role in Algal-Bacterial Interactions, Https://Doi.Org/10.1080/07352689.2016.1172461. 35 (2016) 81–105. https://doi.org/10.1080/07352689.2016.1172461.

[65] S. Das, S. Das, M.M. Ghangrekar, Quorum-sensing mediated signals: A promising multi-functional modulators for separately enhancing algal yield and power generation in microbial fuel cell, Bioresource Technology 294 (2019), 122138, https://doi.org/10.1016/J.BIORTECH.2019.122138.

[66] M. Lakshmikandan, S. Wang, A.G. Murugesan, M. Saravanakumar, G. Selvakumar, Co-cultivation of Streptomyces and microalgal cells as an efficient system for biodiesel production and bioflocculation formation, Bioresource Technology 332 (2021), 125118, https://doi.org/10.1016/J.BIORTECH.2021.125118.

[67] A. Kouzuma, K. Watanabe, Exploring the potential of algae/bacteria interactions, Current Opinion in Biotechnology 33 (2015) 125–129, https://doi.org/10.1016/J.COPBIO.2015.02.007.

[68] N. Yutin, Horizontal Gene Transfer, Brenner's Encyclopedia of Genetics, Second Edition (2013) 530–532, https://doi.org/10.1016/B978-0-12-374984-0.00735-X.

[69] P.J. Keeling, J.D. Palmer, Horizontal gene transfer in eukaryotic evolution, Nat Rev Genet 9 (8) (2008) 605–618.

[70] R.M.M. Abed, J. Köster, The direct role of aerobic heterotrophic bacteria associated with cyanobacteria in the degradation of oil compounds, International Biodeterioration and Biodegradation 55 (1) (2005) 29–37.

[71] L. Chen, X. Wang, D.-J. Lee, Biofilm with highly heterogeneous interior structure for pollutant removal: Cell distribution and manipulated mass transport, Bioresource Technology 343 (2022), 125913, https://doi.org/10.1016/J.BIORTECH.2021.125913.

[72] J. Paniagua-Michel, O. Garcia, Ex-situ bioremediation of shrimp culture effluent using constructed microbial mats, Aquacultural Engineering 28 (2003) 131–139, https://doi.org/10.1016/S0144-8609(03)00011-6.

[73] C.S. Lee, H.S. Oh, H.M. Oh, H.S. Kim, C.Y. Ahn, Two-phase photoperiodic cultivation of algal–bacterial consortia for high biomass production and efficient nutrient removal from municipal wastewater, Bioresource Technology 200 (2016) 867–875, https://doi.org/10.1016/J.BIORTECH.2015.11.007.

[74] A.G. Orfanos, I.D. Manariotis, Algal biofilm ponds for polishing secondary effluent and resource recovery, Journal of Applied Phycology 2019 31:3. 31 (2019) 1765–1772, https://doi.org/10.1007/S10811-018-1731-8.

[75] Y. Lin, L. Wang, K. Xu, H. Huang, H. Ren, Algae Biofilm Reduces Microbe-Derived Dissolved Organic Nitrogen Discharges: Performance and Mechanisms, Environmental Science & Technology 55 (2021) 6227–6238, https://doi.org/10.1021/ACS.EST.0C06915.

[76] H. Zhou, X. Zhao, K. Kumar, T. Kunetz, Y. Zhang, M. Gross, Z. Wen, Removing high concentration of nickel (II) ions from synthetic wastewater by an indigenous

microalgae consortium with a Revolving Algal Biofilm (RAB) system, Algal Research 59 (2021), 102464, https://doi.org/10.1016/J.ALGAL.2021.102464.
[77] P. Maza-Márquez, M.V. Martinez-Toledo, M. Fenice, L. Andrade, A. Lasserrot, J. Gonzalez-Lopez, Biotreatment of olive washing wastewater by a selected microalgal-bacterial consortium, International Biodeterioration & Biodegradation 88 (2014) 69–76, https://doi.org/10.1016/J.IBIOD.2013.12.009.
[78] A. Chavan, S. Mukherji, Treatment of hydrocarbon-rich wastewater using oil degrading bacteria and phototrophic microorganisms in rotating biological contactor: Effect of N: P ratio, Journal of Hazardous Materials 154 (2008) 63–72, https://doi.org/10.1016/J.JHAZMAT.2007.09.106.
[79] J.A.V. Costa, B.C.B.d. Freitas, C.R. Lisboa, T.D. Santos, L.R.d.F. Brusch, M.G. de Morais, Microalgal biorefinery from CO2 and the effects under the Blue Economy, Renewable and Sustainable Energy Reviews 99 (2019) 58–65.
[80] W.Y. Cheah, P.L. Show, J.S. Chang, T.C. Ling, J.C. Juan, Biosequestration of atmospheric CO2 and flue gas-containing CO2 by microalgae, Bioresource Technology 184 (2015) 190–201, https://doi.org/10.1016/j.biortech.2014.11.026.
[81] B. Colman, I.E. Huertas, S. Bhatti, J.S. Dason, The diversity of inorganic carbon acquisition mechanisms in eukaryotic microalgae, Functional Plant Biology 29 (3) (2002) 261.
[82] G. Yadav, S.K. Dash, R. Sen, A biorefinery for valorization of industrial waste-water and flue gas by microalgae for waste mitigation, carbon-dioxide sequestration and algal biomass production, Science of the Total Environment 688 (2019) 129–135.
[83] J. Roostaei, Y. Zhang, K. Gopalakrishnan, A.J. Ochocki, Mixotrophic Microalgae Biofilm: A Novel Algae Cultivation Strategy for Improved Productivity and Cost-efficiency of Biofuel Feedstock Production, Scientific RepoRts | 8 (2018) 12528, https://doi.org/10.1038/s41598-018-31016-1.
[84] E. Koutra, A. Kopsahelis, M. Maltezou, G. Grammatikopoulos, M. Kornaros, Effect of organic carbon and nutrient supplementation on the digestate-grown microalga, Parachlorella kessleri, Bioresource Technology 294 (2019) 122232.
[85] X. Lu, Y. Wan, Z. Zhong, B. Liu, F. Zan, F. Zhang, X. Wu, Integrating sulfur, iron (II), and fixed organic carbon for mixotrophic denitrification in a composite filter bed reactor for decentralized wastewater treatment: Performance and microbial community, Science of The Total Environment 795 (2021), 148825, https://doi.org/10.1016/J.SCITOTENV.2021.148825.
[86] D.H. Cho, R. Ramanan, J. Heo, J. Lee, B.H. Kim, H.M. Oh, H.S. Kim, Enhancing microalgal biomass productivity by engineering a microalgal–bacterial community, Bioresource Technology 175 (2015) 578–585, https://doi.org/10.1016/J.BIORTECH.2014.10.159.
[87] C. Guo, D. Duan, Y. Sun, Y. Han, S. Zhao, Enhancing Scenedesmus obliquus biofilm growth and CO2 fixation in a gas-permeable membrane photobioreactor integrated with additional rough surface, Algal Research 43 (2019), 101620, https://doi.org/10.1016/J.ALGAL.2019.101620.
[88] Z. Yu, H. Pei, Y. Li, Z. Yang, Z. Xie, Q. Hou, C. Nie, Inclined algal biofilm photobioreactor (IABPBR) for cost-effective cultivation of lipid-rich microalgae and treatment of seawater-diluted anaerobically digested effluent from kitchen waste with the aid of phytohormones, Bioresource Technology 315 (2020), 123761, https://doi.org/10.1016/J.BIORTECH.2020.123761.
[89] Y. Wang, Z. Jiang, Z. Lai, H. Yuan, X. Zhang, Y. Jia, X. Zhang, The self-adaption capability of microalgal biofilm under different light intensities: Photosynthetic parameters and biofilm microstructures, Algal Research 58 (2021), 102383, https://doi.org/10.1016/J.ALGAL.2021.102383.
[90] X.Q. Xu, J.H. Wang, T.Y. Zhang, G.H. Dao, G.X. Wu, H.Y. Hu, Attached microalgae cultivation and nutrients removal in a novel capillary-driven photo-biofilm reactor, Algal, Research 27 (2017) 198–205, https://doi.org/10.1016/J.ALGAL.2017.08.028.
[91] M. Bozan, A. Schmid, K. Bühler, Evaluation of self-sustaining cyanobacterial biofilms for technical applications, Biofilm 4 (2022), 100073, https://doi.org/10.1016/J.BIOFLM.2022.100073.
[92] L. Rodrigues de Assis, M.L. Calijuri, P.P. Assemany, T.A. Silva, J.S. Teixeira, Innovative hybrid system for wastewater treatment: High-rate algal ponds for effluent treatment and biofilm reactor for biomass production and harvesting, Journal of Environmental Management 274 (2020), 111183, https://doi.org/10.1016/J.JENVMAN.2020.111183.
[93] A.-M. Lakaniemi, O.H. Tuovinen, J.A. Puhakka, Anaerobic conversion of microalgal biomass to sustainable energy carriers - A review, Bioresource Technology 135 (2013) 222–231.
[94] D. Saidulu, A. Majumder, A.K. Gupta, A systematic review of moving bed biofilm reactor, membrane bioreactor, and moving bed membrane bioreactor for wastewater treatment: Comparison of research trends, removal mechanisms, and performance, Journal of Environmental Chemical Engineering 9 (5) (2021) 106112.
[95] T. Naumann, Z. Çebi, B. Podola, M. Melkonian, Growing microalgae as aquaculture feeds on twin-layers: a novel solid-state photobioreactor, J Appl Phycol 25 (5) (2013) 1413–1420.
[96] Z. Khoobkar, F.P. Shariati, A.A. Safekordi, H.D. Amrei, Performance assessment of a novel pyramid photobioreactor for cultivation of microalgae using external and internal light sources, Food Technology and Biotechnology 57 (2019) 68–76, https://doi.org/10.17113/FTB.57.01.19.5702.
[97] F. Gao, Z.H. Yang, C. Li, G.M. Zeng, D.H. Ma, L. Zhou, A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent, Bioresource Technology 179 (2015) 8–12, https://doi.org/10.1016/J.BIORTECH.2014.11.108.
[98] C. Zamalloa, N. Boon, W. Verstraete, Decentralized two-stage sewage treatment by chemical–biological flocculation combined with microalgae biofilm for nutrient immobilization in a roof installed parallel plate reactor, Bioresource Technology 130 (2013) 152–160, https://doi.org/10.1016/J.BIORTECH.2012.11.128.
[99] E. Posadas, P.A. García-Encina, A. Domínguez, I. Díaz, E. Becares, S. Blanco, R. Muñoz, Enclosed tubular and open algal–bacterial biofilm photobioreactors for carbon and nutrient removal from domestic wastewater, Ecological Engineering 67 (2014) 156–164, https://doi.org/10.1016/J.ECOLENG.2014.03.007.
[100] S. Ekendahl, M. Bark, J. Engelbrektsson, C.-A. Karlsson, D. Niyitegeka, N. Strömberg, Energy-efficient outdoor cultivation of oleaginous microalgae at northern latitudes using waste heat and flue gas from a pulp and paper mill, Algal Research 31 (2018) 138–146.
[101] A. Sathish, R.C. Sims, Biodiesel from mixed culture algae via a wet lipid extraction procedure, Bioresource Technology 118 (2012) 643–647.
[102] H. Cao, Z. Zhang, X. Wu, X. Miao, Direct biodiesel production from wet microalgae biomass of chlorella pyrenoidosa through in situ transesterification, BioMed Research International 2013 (2013) 1–6.
[103] A. Sathish, T. Marlar, R.C. Sims, Optimization of a wet microalgal lipid extraction procedure for improved lipid recovery for biofuel and bioproduct production, Bioresource Technology 193 (2015) 15–24.
[104] E.S.K. Why, H.C. Ong, H.V. Lee, Y.Y. Gan, W.-H. Chen, C.T. Chong, Renewable aviation fuel by advanced hydroprocessing of biomass: Challenges and perspective, Energy Conversion and Management 199 (2019) 112015.
[105] D.R. Vardon, B.K. Sharma, G.V. Blazina, K. Rajagopalan, T.J. Strathmann, Thermochemical conversion of raw and defatted algal biomass via hydrothermal liquefaction and slow pyrolysis, Bioresource Technology 109 (2012) 178–187.
[106] L. Gerber Van Doren, R. Posmanik, F.A. Bicalho, J.W. Tester, D.L. Sills, Prospects for energy recovery during hydrothermal and biological processing of waste biomass, Bioresource Technology 225 (2017) 67–74.
[107] H. Shokrkar, S. Ebrahimi, M. Zamani, Bioethanol production from acidic and enzymatic hydrolysates of mixed microalgae culture, Fuel 200 (2017) 380–386.
[108] J.T. Ellis, N.N. Hengge, R.C. Sims, C.D. Miller, Acetone, butanol, and ethanol production from wastewater algae, Bioresource Technology 111 (2012) 491–495.
[109] I. Valdez-Vazquez, M. Pérez-Rangel, A. Tapia, G. Buitrón, C. Molina, G. Hernández, L. Amaya-Delgado, Hydrogen and butanol production from native wheat straw by synthetic microbial consortia integrated by species of Enterococcus and Clostridium, Fuel 159 (2015) 214–222, https://doi.org/10.1016/J.FUEL.2015.06.052.
[110] A. Constantino, B. Rodrigues, R. Leon, R. Barros, S. Raposo, Alternative chemo-enzymatic hydrolysis strategy applied to different microalgae species for bioethanol production, Algal Research 56 (2021), 102329, https://doi.org/10.1016/J.ALGAL.2021.102329.