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Novel approaches to microalgal and cyanobacterial cultivation for bioenergy and biofuel production

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Growing demand for energy and food by the global population mandates finding water-efficient renewable resources. Microalgae/cyanobacteria have shown demonstrated capacity to contribute to global energy and food security. Yet, despite proven process technology and established net energy-effectiveness and cost-effectiveness through co-product generation, microalgal biofuels are not a reality. This review outlines novel biofilm cultivation strategies that are water-smart, the opportunity for direct energy conversion via anaerobic digestion of N_2-fixing cyanobacterial biomass and integrative strategies for microalgal biodiesel and/or biocrude production via supercritical methanol-direct transesterification and hydrothermal liquefaction, respectively. Additionally, fermentation of cyanobacterial biofilms could supply bioethanol to feed wet transesterification to biodiesel conversion for on-site use in remote locations.

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Introduction

As the world population increases to more than 9 billion people by 2050, food — , clean drinking water — and energy security, as well as climate instability are becoming pressing and interlinked problems with large socio-economic and environmental impacts [1,2]. Algae (micro and macroalgae and cyanobacteria (blue-green algae)) have been heralded as potential saviours, as they can be cultivated on non-arable land, in non-potable nutrient-rich water resources using waste CO_2 and light as key biomass production ingredients [3]. Yet to date, microalgal biofuels production has not transited into reality primarily due to economic competitiveness.

A detailed life-cycle based review focussing on the production potential of biodiesel from microalgae, identified high capital investment requirements, operational costs and biomass loss due to contamination, rather than technological hurdles, as the main reasons [4••]. While energy requirements for dewatering/harvesting are typically included, the large land and water requirements identified by Day *et al.* [5], particularly for open pond-based systems are, however, often not considered. High capital investment requirements can be combated by adopting a staged high value, low volume bio-product strategy with the aim to expand facilities, as capital is raised for actual biofuel production [6,7••]. Reviews by Wijffels *et al.* [8••], Savakis and Hellingwerf [6], and De Bhowmick *et al.* [9] provide detailed outlines of genetic engineering strategies for the enhancement of biofuel potential. Given the resistance to the use of genetically engineered organisms in many nations, particularly in outdoor (uncontrollable) locations, this will not be the focus of this review.

Another aspect receiving little attention, being rarely included even in recent life cycle analyses [10,11], are the fertilisation requirements of microalgae for optimal growth, because recycling of nutrient-rich waste waters from the downstream biomass to fuel processing pathways [7••] or collocation with nutrient-rich water resources, such as water treatment plants (e.g. [12,13] and other references in this volume) is envisaged. Typical industry process diagrams rarely align production fertilisation — with areal requirements, leading to process diagrams that may be implementable at pilot-scale or for on-site supplies only, but fail to deliver at product market scales.

Given the above, this review will briefly touch on microalgal strain selection, that is, biomass biochemical profile requirements, in light of extraction/fuel production technology and their impacts on biomass dewatering requirements. It will also discuss alternative production pathways, incorporating alternatives for energy production and fertiliser recycling.

The bio-products trap — hindrance or facilitator for fuel production?

Microalgae and cyanobacteria have an undeniably high industrial potential for high value, low volume bio-product markets, as demonstrated by their contribution to the highly lucrative pigment and food supplement markets. The production potential for microalgal products has been reviewed in depth in recent years (e.g. [14–16]), most highlighting the need for process integration of waste recycling for economic production of biofuels [17••] and co-production of fine chemicals [18]. It is noteworthy though that, despite workable net energy and cost-effectiveness of this multiple co-product and by-product approach [17••], microalgal biofuels are still not being produced at any scale. This could be indicative of a catch 22 situation where high value products could drive the economics and investment at the expense of progressing to low-value biofuel production until markets are saturated. One aspect mentioned for targeted high value bio-product markets, but receiving little attention, is the necessity for cultivation of specific strains, which have the obligatory biochemical profile — yields and — productivities to meet required productivities [19]. This has flow on effects on the economics of such production facilities, due to either more cost-prohibitive system requirements (e.g. closed systems) and/or impacts of contamination (e.g. open raceway ponds). This review investigates the possibility of a direct biofuels approach by integrating waste recycling, energy generation and waste product-derived co-products.

System considerations

To date, open raceway or hybrid system production of microalgal biomass appears to be the general consensus for economic biofuel generation [20], but, irrespective of system, the requirement for water movement to keep the biomass homogenously resuspended for light exposure and dewatering/harvesting of relatively dilute biomass (often <1 g dry weight (DW) L^{-1}) can increase the cost of the operation, both in terms of capital and energy expenditures, for example, 0.21 kWh $kg_{biomass}{}^{-1}$ for raceway operation and 0.42 kWh $kg_{biomass}{}^{-1}$ for centrifuge-based dewatering/harvesting [21]. In an interesting life cycle analysis, Handler *et al.* [21] investigated energy requirements for different systems (stirred tank secondary treated sewage and raceway) integrated with different biofuel processing pathways, fast pyrolysis (RTP™, Rapid Thermal Pyrolysis for the former) and oil extraction followed by hydro-processing for the latter cultivation approach and created a novel scenario where raceway cultivation of biomass was coupled with fast pyrolysis. Despite potential greenhouse gas emission savings of ~85% compared to petroleum petrol production, switching dewatering from settling to dissolved air-floatation (DAF) eroded the greenhouse gas emission savings basis by more than 50%.

A novel and recently more investigated cultivation strategy is biofilm cultivation of microalgae [22,23••]. These systems have traditionally been used for remediation of waste waters, probably best known as algal turf scrubbers, but a serious link for the commercial production of microalgal biomass has been made only recently [22]. Microalgal biofilm cultivation avoids large energy expenditure for mixing and dewatering/harvesting (Table 1), as the biomass scraped of a cultivation surface yields a paste with a similar total solid content to that obtained by centrifugation. Furthermore, as the algal biofilm is separated from the air by only a thin layer of water, irrespective of system design (Figure 1) [22], carbon dioxide and light utilisation is much improved [24]. Algal species choice in these systems is positively correlated to the hydrophobicity of the cell surface, providing superior attachment to the cultivation substratum [23••]. Cultivation surface productivity of these systems typically range from 2 to 6 g DW m^{-2} day^{-1}, while system footprint biomass can vary considerably based on design from 5–10 to 46–80 g DW m^{-2} day^{-1}, with rotating and vertical systems showing the highest biomass productivities even in pilot-scale operation [23••]. Based on algal turf scrubber species analyses for waste water treatment, freshwater green microalgal species grow readily has biofilms [23••] and the successful cultivation of the nitrogen-fixing and self-settling cyanobacterium *Tolypothrix* sp. was also recently shown for outdoor cultivation in the semi-arid tropics [25]. The biofilm cultivation approach when integrated with biomass to fuel/energy conversion scenarios can yield novel theoretical strategies for biofuel/bioenergy using microalgae/cyanobacteria.

Biofilm-integrated microalgal/cyanobacteria biofuel/bioenergy production

The various microalgal cultivation biofilm strategies are described in Box 1, where considerations of footprint, water loss and suitability for different applications are detailed.

Many microalgae are capable to grow as biofilms in a perfused biofilm cultivation system, providing environmental conditions are sufficiently humid [22]. A scenario for self-sufficient perfusion biofilm-generated microalgal

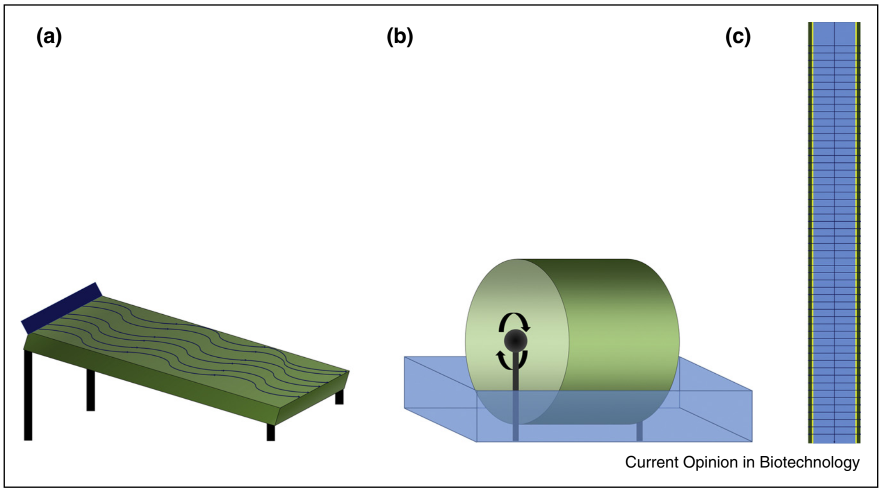
Table 1

Comparison of energy and water requirements of open ponds (OP), vertical flat panel (VFP) and biofilm cultivation systems (BF) for biomass cultivation and dewatering/harvesting

Parameter	OP	VFP	BF
Biomass areal productivity [g m^{-2} d^{-1}]	48[a]	68[a]	2–80[b,c]
Energy for cultivation [kWh bbl^{-1}]	333[a]	294[a]	N/A
Water consumption [m^3 bbl^{-1}]	312[a]	34[a]	178[d] 22[e]
Energy for harvesting/dewatering			
Centrifugation [kWh bbl^{-1}]	1352[a]	–	–
Chitosan flocculation [kWh bbl^{-1}]	–	135[a]	–
Chamber press filtration [kWh bbl^{-1}]	1190[a]	–	–

[a] Ref. [48•].
[b] Ref. [22].
[c] Ref. [23••].
[d] Based on [62] for a horizontal ATS.
[e] Based on [32•] for a vertical water troph-positioned rotating biofilm reactor; bbl: barrel of oil (159 L).

Figure 1



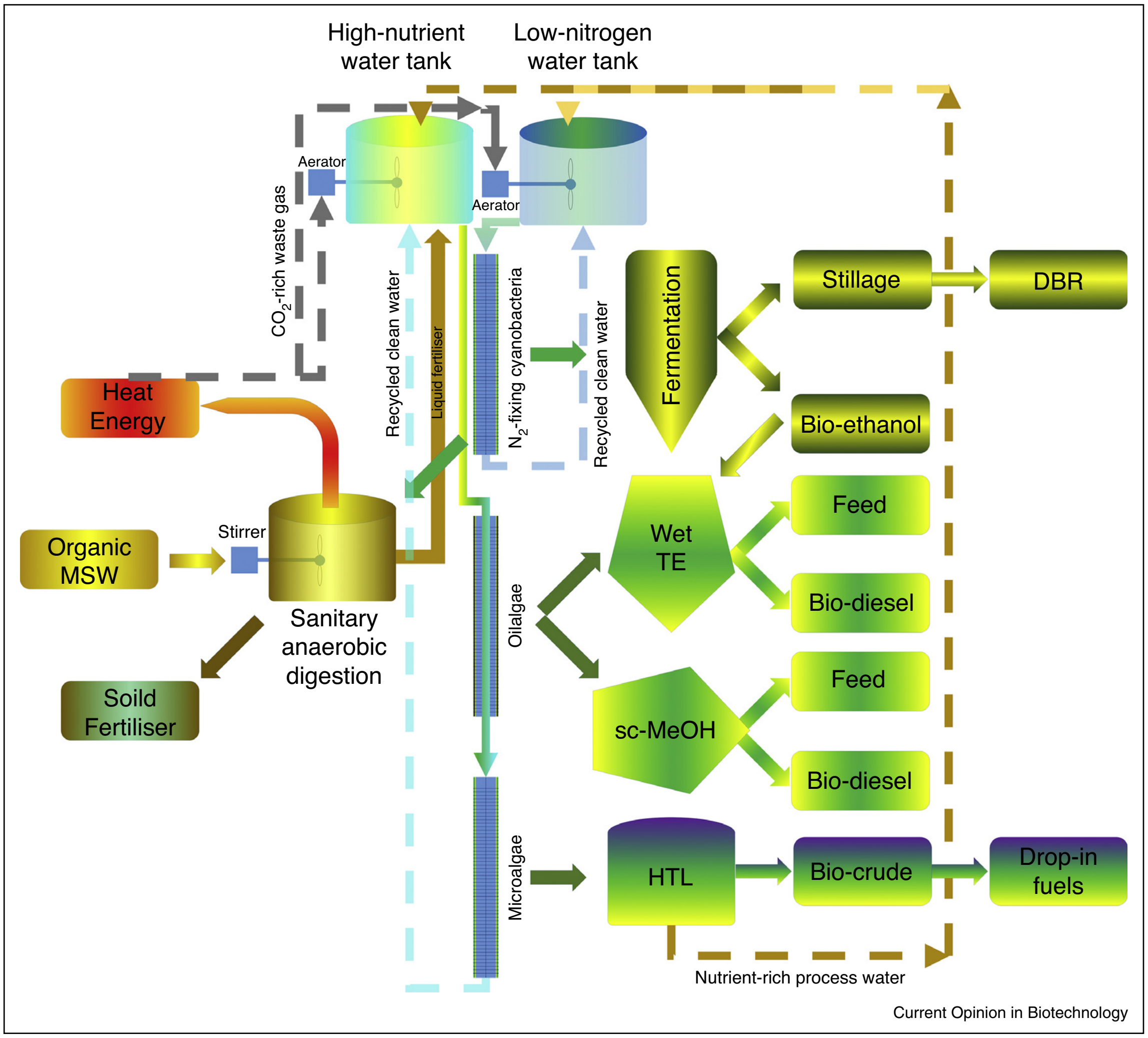
Microalgal biofilm cultivation systems. **(a)** Algal turf scrubber, **(b)** rotating intermittently submerged drum system, which is ideally suited to remediate existing nutrient-rich water bodies (e.g. blue basin symbolises water treatment pond) and **(c)** perfused vertical algal biofilm design, where the yellow line symbolises the nutrient-permeable and water permeable but cell-impermeable cultivation substrate mounted on the nutrient-rich water conducting material (blue interior) and the green line, the algal surface biomass. The dark blue thin lines indicate the water flow into the biofilm and out through the water-conducting material.

biofuel production is shown in Figure 2, which assumes production in a humid high light agricultural location using anaerobic digestion (AD) as the energy source to drive production and biomass conversion processes. N_2-fixing cyanobacterial biomass are starch-rich and protein-rich and therefore ideal for fermentation to bioethanol [34–38]. The use of N_2-fixing cyanobacteria has rarely been considered, although fertilisation requirements would be limited to the provision of phosphate and iron. Our studies show that the non-toxic, tropical N_2-fixing cyanobacterium *Tolypothrix* sp. can be grown successfully in outdoor biofilms [25] yielding comparable growth profiles when grown with or without inorganic nitrogen (Velu, Cirés and Heimann, unpublished). Among the naturally oil-rich microalgae are benthic diatoms that readily form thick biofilms and have been more the focus of antifouling research [39–41], while microalgae can be produced with increased oil content by switching from nutrient-sufficient to limiting conditions [42–46]. This biomass can be converted to biodiesel using either wet transesterification, potentially accelerated by pressure-assisted solvent extraction (not shown) [47], or super-critical methanol extraction and transesterification (sc-MeOH) (Figure 2). Using the process criteria for 10 000 MJ of algal biodiesel in a life cycle analysis by Brentner *et al.* [48•], biofilm cultivation would save 616 (paddle wheel, aeration and outflow pumping) and 2500 kWh on centrifugation, while using sc-MeOH would require 6080 MJ compared to 8180, 30 280 and 47 200 MJ using supercritical CO_2-, drying, pressing and hexane-extraction, both followed by transesterification, or sonication-direct transesterification with acid hydrolysis, respectively. The same study shows energy-credit (2176 kWh $10GJ^{-1}{}_{biodiesel}$) and nutrient credit (1176 kWh $10GJ^{-1}{}_{biodiesel}$) generated by subjecting the extracted microalgal biomass to AD. Alternatively though, N_2-fixing cyanobacteria could be grown purposefully for AD energy generation (Figure 2), leaving the extracted biomass as a feed co-product. One of the very few microalgal biodiesel engine performance

Box 1 Microalgae biofilm cultivation systems

Microalgae biofilm cultivation systems can be defined by the submersion characteristics and the flow of nutrient-rich water (Figure 1a–c). Irrespective of design, supply of CO_2 is much more effective due to the thin nature of the water film allowing more effective diffusion compared to suspension-based cultivation approaches [26•]. In algal turf scrubbers (ATS) (Figure 1a), the biomass grows permanently submerged on an angled horizontal surface [22,23••]. These systems have been used successfully for the remediation of nutrient-rich waste waters in aquaculture, large aquaria and water treatment plants [27–31]. Due to the angled horizontal nature of ATS, the systems are ideal for implementation in more temperate regions, but system footprint and evaporative water loss is greater than in vertical designs [32•] (Table 1). Moreover, the microalgae to be cultured must be tolerant to high light. Biofilms in rotating systems (Figure 1b) on the other hand are only intermittently submerged, but have achieved highest biomass productivities [23••,32•] and are ideally implemented in places with nutrient-rich waste water ponds, such as water treatment plants [33•]. Consequently, evaporative water loss is dictated by the nature of the water surface area of such ponds. A relatively new design are perfused microalgal biofilm systems, where the algae are grown on a nutrient-permeable and water-permeable surface supplied via water-conducting material from behind (Figure 1c) [22]. System arrangements are typically vertical with small system footprints and spacing of the systems affords light dilution. Evaporative water loss will depend on environmental humidity; the system characteristics make them suitable for cultivation in high light and high humidity locations, such as the tropics [22,25].

Figure 2



Schematic of a self-sufficient perfused microalgal/cyanobacterial biofilm cultivation for biofuel production. The scenario assumes limited nutrient-rich or organic municipal waste availability in a rural, humid and high-light agricultural setting. N_2-fixing cyanobacteria are cultivated in low-nitrogen, phosphate-sufficient and CO_2-supplemented water to provide biomass for anaerobic digestion (AD) and bioethanol production via fermentation. Stillage provides dried biomass residue (DBR) as a feed supplement, while the bioethanol could be used to assist wet transesterification (Wet TE) of the biomass of oil-rich algal (oilalgae) for the production of biodiesel and feed. Alternatively, oil-rich algae could yield biodiesel and feed via supercritical methanol (sc-MeOH) extraction and transesterification. Energy for required drying of input (Wet TE) and feed (fermenter DBR-derived, Wet TE-derived and sc-MeOH-derived) outputs and general energy requirements of the individual processes, including solvent recycling (not shown) would be supplied via AD. Nutrient-rich water return has only been considered for microalgal biomass conversion to biocrude via hydrothermal liquefaction (HTL) and the liquid leachate of the AD. The HTL-derived biocrude requires refining (deoxygenation and denitrification) for drop-in biofuel generation, which is assumed to take place at a sufficiently large external refinery. Finally, the residual solids from the AD would themselves be suitable as a fertiliser product.

studies showed that even the heterotrophically-produced dino flagellate *Crypthecodinium cohnii* with an extraordinarily high long chain polyunsaturated fatty acid (LC-PUFA) content, which would theoretically be detrimental due to adverse impacts on biodiesel quality [49], was suitable at current and future blending rates (B5 and B20), respectively [50•]. It can therefore be expected that diatoms, naturally rich in the LC-PUFA, eicosapentaenoic acid (EPA), would yield similar biodiesel quality and engine performance results.

At present, the most frequently studied microalgal biofuel production route is hydrothermal liquefaction (HTL), as the water itself acts as a solvent at subcritical temperature settings, avoiding costly dewatering infrastructure and energy expenditure [51•,52–59]. Although other thermochemical biofuel routes like torrefaction, slow and fast pyrolysis, and gasification are also being intently studied for algal fuel and energy potential, these are not discussed further here due to higher energy requirements [21,51•,52–55]. Cultivation of microalgae/cyanobacteria in perfusion biofilms will require dilution with low nutrient water prior to HTL to meet the maximum solid loading of no more than 10 wt.% [60]. A study by Biller *et al.* [61•] showed that HTL biocrude yields from green microalgae and the cyanobacterium *Spirulina* were comparable irrespective of large differences in lipid content. The same study also determined that HTL process water required significant dilution (200–400×) to sustain microalgal growth depending on species and HTL process conditions. Even though the perfused biofilm cultivation system offers switching of water supply to low nutrient water prior to harvest, this can have implications for the process outlined in Figure 2 with regards to matching HTL process water recycling with additional liquid fertiliser generated via AD. Should the outlined process, if energy requirements are to be met for biomass to biofuel processing, generate too much nutrient effluent, the opportunity does exist for liquid fertiliser co-product development using AD leachate and/or blending of HTL process water with nutrient-poor water.

Conclusions

Biofilm cultivation of microalgae and cyanobacteria offer novel water-smart biomass production pathways, which can readily feed into currently explored biofuel processing pathways. While some growth data exist, more detailed growth behaviour across seasons for the different systems, especially perfusion-based biofilm systems, are required to calculate area footprint and cultivation energy requirements with any certainty. Based on available biomass and biofuel process data, the time has come to translate theory into practice. This can be achieved at small scales initially aiming to meet the biofuel and feed demand in remote, rural locations generating required energy to drive cultivation, extraction and refining processes via anaerobic digestion of N_2-fixing cyanobacterial biomass. Ideally, locations should be chosen based on future expansion opportunity, which would also provide a strategy to create new agricultural industries.

Conflict of interest

Nothing declared.

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