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Recent advances in engineering fast-growing cyanobacterial species for enhanced CO₂ fixation

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Atmospheric CO₂ removal (CDR) is a fundamentally endergonic process. Performing CDR or Bioenergy with Carbon Capture and Storage (BECCS) at the gigatonne scale will produce a significant additional burden on the planet's limited renewable energy resources irrespective of the technology employed. Harnessing photosynthesis to drive industrial-scale CO₂ fixation has been of significant interest because of its minimal energy requirements and potential low costs. In this review, we evaluated the thermodynamic considerations of performing atmospheric carbon removal using microalgae and cyanobacteria versus physicochemical processes and explore the implications of these energetic costs on the scalability of each respective solution. We review the biomass productivities of recently discovered fast-growing cyanobacterial strains and discuss the prospects of genetically engineering certain metabolic pathways for channeling the fixed carbon into metabolic 'carbon sinks' to further enhance their CO₂ capture while concurrently extracting value. We share our perspectives on how new highly productive chassis strains combined with advanced flux balance models, essentially coupling synthetic biology with industrial biotechnology, may unlock more favorable methods for CDR, both from an economic and thermodynamic perspective.

KEYWORDS

synechococcus, cyanobacteria, carbon removal, CO₂ capture, genetic manipulation

1 Introduction

The world produces 40 billion tonnes/year (T/y) in anthropogenic CO₂ emissions (Friedlingstein et al., 2023). Based on estimates from the IPCC, approximately 10 billion tonnes of carbon removal is required annually between now and 2100 to meet the requirement to limit global warming to 1.5°C (IPCC, 2018). As of 22nd March 2024 a total of \$2.34 billion USD was spent on carbon removal credit purchases for 5.8 million tonnes of CO₂ removal, of which only 5.1% have been delivered (CDR, 2024). In order to meet these requirements, carbon removal must increase by four orders of magnitude. Direct Air Capture (DAC) and Bioenergy Carbon Capture & Storage (BECCS) have the largest market capitalization, representing 19 and 64% of the purchases, respectively (CDR, 2024). At the gigatonne scale, the additional renewable energy that would be required to use conventional CO₂ capture technologies poses a major feasibility concern (McQueen et al., 2021; Soeherman et al., 2023; Prats-Salvado et al., 2024).

Earth receives approximately 4.3×10^{20} joules of solar irradiance per hour, providing more energy in 1 h than all of the energy consumed by human activity in a year (Lewis and Nocera, 2006). However, the vast majority of this solar capacity remains underutilized (Blankenship et al., 2011). An estimated 115 billion tonnes of CO₂ is fixed by photosynthesis annually (Field et al., 1998). Despite the great interest in BECCS, growing fuel crops such as switchgrass to harness photosynthetic productivity at industrial scale still poses a major disadvantage in the areal requirement for arable land. Cyanobacteria and microalgae boast a significantly greater areal productivity and CO₂ fixation rate and can yield ~4 fold higher biomass (Subramanian and Sayre, 2022), and 15-300-fold more oil production than terrestrial plants (Chisti, 2007; Hempel et al., 2012; Rehman et al., 2022). Furthermore, cultivation of microalgae and cyanobacteria can be conducted in open ponds or closed vessels on non-arable land, thereby avoiding direct competition for limited agricultural land for the production of biomass (Righelato and Spracklen, 2007). Cyanobacteria contain CO₂ concentration mechanisms (CCM), most notably the carboxysome, that allow higher carbon dioxide fixation efficiencies compared to terrestrial plants and macroalgae, leading to higher yields (Savir et al., 2010). Moreover, certain cyanobacterial species can be more rapidly and precisely genetically modified than plants and macroalgae (Gale et al., 2019), suggesting that strains could be further improved to increase biomass yields, aided by recent advances in genetic engineering tools such as CyanoGate (Vasudevan et al., 2019). Despite attempts to utilize microalgae for biofuel production in the 2000s and early 2010s, these initiatives largely faltered due to inadequate biomass yields and the substantial expenses linked to lipid extraction techniques (Abo et al., 2019; Venkata Subhash et al., 2022). Successful implementation of BECCS is therefore dependent on identifying and utilizing faster growing and higher biomass producing strains.

Recently discovered fast growing cyanobacteria, including *Synechococcus* sp. PCC 11901 and *Cyanobacterium aponinum* UTEX 3222, have the highest biomass productivity observed among known cyanobacterial and microalgal species (Włodarczyk et al., 2020; Schubert et al., 2023). *Synechococcus* sp. PCC 11901 is of particular interest due to its natural competence, fast growth (doubling times of less than 2.5 h) and very high productivity (32.6 g/L in 14 days) when cultured under industrially relevant CO₂ concentrations of ~5% (e.g., CCGT natural gas power stations). This could potentially make it suitable for cultivation for compounds/biomass production and carbon capture including BECCS, as well as atmospheric carbon removal (Mills et al., 2022; Victoria et al., 2023). Furthermore, other strains of *Synechococcus* sp. have been well-characterized and possess traits of interest such as native production of commercially valuable compounds (e.g., phycocyanin), fast growth and natural competence, making them potentially useful chassis (Goodchild-Michelman et al., 2023).

Nevertheless, the scalability of employing photosynthesis for mass-scale carbon removal is hindered by inherent efficiency limitations concerning the flux balance at a subcellular level, with only 1–3% of the energy in incident photons being utilized for biomass production (Hann et al., 2022). This poses both an unfortunate bottleneck, as well as an opportunity to make major efficiency gains if carbon flux can be partitioned to pathways with faster reaction kinetics – lending credit to the idea of metabolically engineered carbon sinks (Oliver and Asumi, 2015; Zhang et al., 2017; Hann et al.,

2022). Hence, alongside process improvements, at a physiological level an overall improvement in the efficiency of factors such as biological light capture and CO₂ assimilation may lead to productivity thresholds needed to make carbon sequestration at an industrial scale possible while limiting land use and CAPEX (de Vree et al., 2015; Even et al., 2022; Klein and Davis, 2023). Potentially, cyanobacterial biofilms and immobilization in polymer-based compounds could be utilized which has the advantages of consuming less water and allowing high-density growth but in the case of immobilization, cells can no longer be harvested for biomass (Caldwell et al., 2021). Moreover, not all cyanobacteria readily form biofilms.

In this review, we discuss the application of metabolic engineering for improving carbon sinks or other desirable traits to generate fast growing cyanobacterial strains with improved CO₂ fixation, thermotolerance and more efficient light-harvesting capacity. A ‘carbon sink’ in this review refers to high flux metabolites or carbon dense compounds that need further downstream processing for permanent sequestration. We categorize each respective approach and evaluate where there is greatest potential for future studies. First, we describe the comparative thermodynamic efficiency limits of performing carbon capture through biological sequestration versus conventional physicochemical methods for atmospheric carbon removal. We illustrate the magnitude of the additional energetic burden posed on the planet when performing carbon capture, irrespective of technology, at the gigatonne scale that is being suggested by the IPCC (Calvin et al., 2023). Second, we conclude that CO₂ separation is a fundamentally endergonic process and therefore carbon capture at the gigatonne scale must either become fully powered by renewable energy that has the potential to scale proportionally with CDR demand, or it must be done through a self-powered process like photosynthesis where there is no additional energetic burden. Third, we review the current feasibility of deploying cyanobacteria for BECCS and CDR at scale. Finally, we examine the recent advances in using genetic engineering to further enhance the rate of CO₂ fixation in recently discovered fast-growing cyanobacteria that have opened up new possibilities in making this an economically compelling solution capable of scaling rapidly in the near future.

2 Thermodynamics underpinning photosynthesis for CO₂ fixation

Both living and non-living systems are bound by the laws of thermodynamics that bear significant relevance to any discourse assessing the viability of performing CO₂ sequestration at the gigatonne scale. Animated matter can be considered to be a self-assembling open system, wherein, a separation of substances is achieved between the cell and its environment at the most fundamental level (Popovic, 2019). At the system level, biological life can be conceived as the maintenance of a disequilibrium state against its surroundings (Weber, 2010), increasing its own order and adding entropy of the environment in the process. As the organism creates ordered structures, utilizes energy, divides and responds to the environment, these reactions all have thermodynamic parameters, like any chemical reaction. It is therefore necessary to derive the thermodynamic properties of the reactions that convert CO₂ in the environment into biomass, including the Gibbs free energy, entropy and enthalpy calculations, to arrive at a proper comparison against

chemical carbon capture technologies. Furthermore, these calculations lend mathematical evidence to why biology may be uniquely resourced to perform the synthesis of more complex molecular structures from CO₂ fixation—thus introducing the rationale for metabolic engineering and carbon sinks.

2.1 Second-law efficiencies dictate the minimum energetic requirements for performing carbon removal

Based on the second law of thermodynamics, there is a minimum theoretical energy required to perform separation of CO₂ (0.04%) from the air irrespective of the energy efficiency for a given process (House et al., 2011). The theoretical minimum is the net change in exergy of a closed system for a pure separation process. Assuming that the conditions are isothermal and isobaric, this net change in exergy is equal to the change in Gibbs free energy (Equation 1). The minimum work required can be calculated to be 19,385.63 J/mol or 122.35 kWh/T CO₂. Because of reversibility, it is impossible in the practical sense to be operating at the theoretical minimum. Therefore, most separation processes operate at anywhere between 2.5-fold (for desalination via reverse osmosis) to 20-fold for DAC (Dahmus and Gutowski, 2007). Separation systems typically require significantly more work done compared to the minimum thermodynamic work required—this ratio is termed the ‘second law efficiency’ which in carbon capture is approximately 5% (House et al., 2011).

$$\Delta G = \Delta H - T\Delta S$$

$$\Delta S = nR \ln \left(\frac{C_1}{C_2} \right)$$

$$w_{\min} = -T\Delta S$$

$$W_{\min} = -T \sum_{k=2} R \ln \left(\frac{C_1}{C_2} \right)$$

$$W_{\min} = -T \left(R \ln \left(\frac{100}{0.04} \right) + R \ln \left(\frac{100}{99.96} \right) \right)$$

$$W_{\min} = -19.39 \text{ kJ} / \text{mol} \quad (1)$$

Equation 1: the theoretical minimum work to concentrate CO₂ from ambient air at 0.04% concentration is 19.39 kJ/mol. Where C denotes the molar concentration of substance k in an ideal gas, ΔG denotes change in Gibbs free energy, ΔH denotes change in enthalpy and R equals ideal gas constant.

Assuming a 5% second-law efficiency, approximately 388 kJ/mol of energy (2,500 kWh/ T CO₂) is needed to separate CO₂ from air irrespective of technology. Previous techno-economic modeling of the cost of DAC systems at scale concluded that this is likely to be only carbon negative if powered by non-CO₂ emitting energy sources

(House et al., 2011). Taken one step further, we ought to also ask what the opportunity cost is for each additional kWh of renewable energy spent on performing atmospheric carbon removal at this energetic cost for carbon capture compared to the amount of CO₂ emission reduction that could be achieved if supplied to the grid to prevent further emissions from combustion of fossil-derived resources. Assuming a 10% second-law efficiency, approximately 1,250 kWh/T CO₂ is required—this is what most second generation DAC technologies are projecting to be able to perform at scale using metal-organic frameworks (MOFs; Kumar et al., 2015; Darunte et al., 2016). When capturing from concentrated emission sites such as biogenic power stations emitting 5% CO₂ in the flue gas for BECCS-type applications, the minimum work drops to 47.6 kWh/TCO₂, making this process significantly more favorable. It is worth noting that the concentration has been increased 125-fold compared to the previous example, but the minimum work against entropy has been reduced by only 60%.

$$W_{\min} = -T \left(R \ln \left(\frac{100}{5} \right) + R \ln \left(\frac{100}{95} \right) \right)$$

$$W_{\min} = -7.55 \text{ kJ} / \text{mol} \quad (2)$$

Equation 2: The theoretical minimum work to concentrate CO₂ from biogenic emission sources (BECCS) at 5% concentration is 7.55 kJ/mol. Where C denotes the molar concentration of substance k in an ideal gas, ΔG denotes change in Gibbs free energy, ΔH denotes change in enthalpy and R equals the ideal gas constant.

In a recent multi-component technoeconomic analysis for Direct Air Capture and Storage (DACCS), the process-specific thermodynamic minimum energy required was calculated to be 964, 1,222 and 667 kWh/TCO₂ for liquid solvent, solid sorbent and CaO ambient weathering technologies, respectively (Table 1). This is 5–10 fold higher than the thermodynamic minimum calculated in Equation 1 for the separation of CO₂ from air (Sievert et al., 2024).

The IPCC targets 10 Gt/year or 420–1,100 Gt of carbon removal in total before 2,100 (5.6–14.7 Gt/year; Carbon Removal | World Resources Institute, 2023; Calvin et al., 2023). Therefore, scaling any atmospheric carbon removal technology at 1,230 kWh/T CO₂ (194 kJ/mol), assuming that 10% second-law efficiency is achieved, by 10 Gt/year, results in energy consumption of 12,300 TWh/year (43% of global electricity production). Even assuming that we perform CDR at the thermodynamic minimum of 19.4 kJ/mol at the 10 Gt/year scale, we approach an energy consumption of 1,230 TWh/year, approximately 4.3% of current global energy production of 28,661 TWh/y (based on 2022 estimates; Ritchie et al., 2024). These figures exclude the additional energy that would be required for any processes associated with the permanent storage or compression of the captured CO₂.

Approximately 61% of global energy production is from coal, oil and gas (Ritchie et al., 2024). Therefore, CDR would consume 4.3% of the world's annual electricity production to capture 25% of annual anthropogenic CO₂ emissions. Therefore, atmospheric carbon removal is only environmentally practical if powered by low-carbon or renewable energy (McQueen et al., 2021). However, since renewable energy can potentially be better spent decarbonizing the energy mix,

TABLE 1 Comparing thermodynamic minimum for atmospheric carbon removal.

Thermodynamic energy requirement (kWh/T CO ₂)	Liquid solvent	Solid sorbent	CaO weathering	References
Minimum energy for separation from air (as per the Equation 1)	123	123	123	This work
Minimum energy for separation from air*	964	1,222	667	McQueen et al. (2021); Sievert et al. (2024)
True energy requirement	1839	2,997	2,500	McQueen et al. (2021); Young et al. (2023)

*Thermodynamic minimum calculated based on the sum of processes requiring electrical and heating energy in Sievert et al.

TABLE 2 Comparison of the changes in enthalpy, entropy and free energy of combustion for *Chlorella* sp. biomass (reaction 1, Popovic, 2019), *Synechococcus* sp. biomass (reaction 2) and for DAC under standard conditions.

Process	-ΔH°/ kJ/mol_CO ₂	ΔS°/J/(mol_CO ₂ .K)	-ΔG°/kJ/mol_CO ₂
CO ₂ captured by <i>Chlorella</i> sp. biomass (Reaction 1)	551.7	4.4	553.01
CO ₂ captured by <i>Synechococcus</i> sp. biomass (Reaction 2)	274.74	≈4.4*	276.05
Atmospheric carbon removal (thermodynamic minimum)	0	65.03	19.39

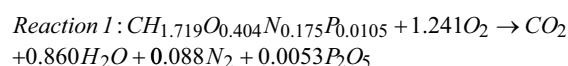
*The entropy change for *Synechococcus* sp. biomass was not readily available in literature, nor was the information necessary to estimate it. Since reaction 1 and reaction 2 are similar in nature and both reagents and products start and end in similar states, and using the fact that entropy is a state function, we can infer that the entropy change for reaction 2 is in the order of magnitude as that of reaction 1 and assume the same value for the sake of a comparison analysis. In this table we compare the values from Equation 1 with the empirical values obtained from *Chlorella* sp. biomass and estimated for *Synechococcus* sp. biomass. This approach allows us to see the energy levels involved in the interactions that both approaches have with CO₂.

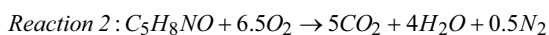
It has been widely suggested that gigatonne-scale atmospheric carbon removal ought to come after a near-complete renewable energy transition for it to be justifiable (House et al., 2011). One study reported that, assuming a 20% second law efficiency of atmospheric CO₂ removal at 2,500 kWh/T CO₂, powering such a technology with 1 kWh of energy would at best, capture approximately the same amount of CO₂ that is being emitted by a coal power station to produce 1 kWh. Hence the reason why photosynthesis becomes an interesting approach if it can be engineered (Soeherman et al., 2023). It was concluded from a technoeconomic analysis by Wilcox and colleagues: "Even if the region is no longer building new high-carbon plants but is still running its old ones, shutting down these old plants in favor of low-carbon energy sources is likely to be a better use of these low-carbon sources than powering DAC. Only when the region's electricity system is nearly completely decarbonized, do the opportunity costs of dedicating a low-carbon electricity source to DAC disappear." (McQueen et al., 2021). Considering the energy alone involved directly in separating CO₂, the cost makes performing atmospheric carbon removal difficult to justify from an efficiency

perspective. In contrast, photosynthesis is energetically additional and therefore does not compete for the limited global renewable energy supply to perform CO₂ removal.

2.2 Photosynthesis performs an enthalpy change in addition to CO₂ separation

In contrast to CDR, carbon fixation in photosynthetic organisms is powered by ATP and NADPH produced by a self-repairing electron transport chain in the thylakoid membrane (Lea-Smith and Hanke, 2021). Furthermore, CO₂ capture is inextricably coupled to CO₂ fixation into organic compounds with higher energy states (Popovic, 2019). These comprise predominantly lipids, carbohydrates and proteins that constitute what is broadly termed as 'biomass'. The stoichiometry of the formation of biomass from its constituent reactants can be represented in the equations below where Reaction 1 denotes the combustion of *Chlorella* sp. biomass while Reaction 2 denotes the combustion of *Synechococcus* sp. biomass (Nagarkar et al., 2004; Quintana et al., 2011; Popovic, 2019; see Appendix 1.3). The laws of thermodynamics render any work done against entropy to be permanently irrecoverable. Therefore, it is of paramount importance to know how much work is done against entropy in comparison to the work done to an enthalpy change, in a reaction that separates CO₂ as gas versus a biological system wherein CO₂ is captured and converted into biomass. Next, we draw on values for enthalpy and entropy of formation for microorganisms estimated experimentally by Popovic et al., along with Hess' law to determine the enthalpy of combustion values under standard conditions (Popovic, 2019). To estimate the molecular composition of *Synechococcus* sp. biomass based on the percentage of protein, lipid, and carbohydrate content, we use their typical composition as a basis (see Appendix 1.1) for the calculation leading to the formula C₅H₈NO (Nagarkar et al., 2004). The calorific value of microalgal and cyanobacterial biomass in the literature ranges from 2.8 kJ/g to 21 kJ/g dry cell weight (Scragg et al., 2002; Nagarkar et al., 2004). We have taken a conservative estimate, given the experimental calorific value of 2.8 kJ/g dry cell weight (DCW) in Nagarkar et al., for *Synechococcus* sp. biomass. We then calculated the enthalpy of combustion, which is presented in Table 2 (see Appendix 1.2 for calculation details).





Since the energetic requirement for biological sequestration includes the enthalpy change, the total amounts to 274 kJ/mol of CO₂ of additional work (for *Synechococcus*) that has been performed by biology to convert CO₂ into a higher energy state (biomass), equivalent to an energy gain of 1,729 kWh/T CO₂, whereas there is no enthalpy change in the pure separation of CO₂ from air.

From Table 2, we can deduce that significantly greater energy is required to capture each mol of CO₂ using biological sequestration as biomass compared to a chemical process separating CO₂ from air at the thermodynamic minimum (derived from Equation 1). However, most of this energy is consumed by an enthalpy change, meaning that this energy is not lost from the system and therefore, cyanobacteria and microalgae serve as extraordinarily efficient biological factories. To determine whether performing carbon removal makes sense at a fundamental reaction level, these values do not consider the practical energetic costs of CO₂ transport, compression and storage for atmospheric carbon removal, nor the energetic costs associated with extraction, cultivation and processing of a biological system. It should also be noted that atmospheric carbon removal can be done to produce CO₂ as a substrate for manufacturing processes and that CO₂ has a very low standard enthalpy state of formation at −393.5 kJ/mol (Wang et al., 2019). A pure stream of compressed CO₂ generated from atmospheric carbon removal can still technically undergo an equivalent enthalpy change through a downstream chemical process such as Fischer-Tropsch, to produce hydrocarbons or organic compounds (Male et al., 2021; Soeherman et al., 2023). However, this is performed at a significant energetic penalty which must be calculated and added on to the Gibbs free energy change for the thermodynamic minimum for atmospheric carbon removal, if we are to make a true comparison of the end-to-end energetic efficiencies of performing biomanufacturing from photosynthesis against chemically converting CO₂ after performing direct air capture (Male et al., 2021; Soeherman et al., 2023). Since the enthalpy change undergone by *Synechococcus* sp. (Table 2) is equivalent to a gain in chemical potential energy (at isothermal conditions), engineering metabolic pathways to favor carbon flux inside a cell toward biosynthesis enables much of this energy to be recovered in the extractable compound of interest, which may facilitate generating economic value from carbon. Hence, unlike conventional carbon capture technologies, with biological sequestration there is a theoretical potential to generate revenues that can offset costs of capture because an enthalpy change has occurred (Zhang et al., 2017; Beal et al., 2018; Onyeaka et al., 2021).

2.3 The low entropic penalty of biomass conversion implies possible room for improvement

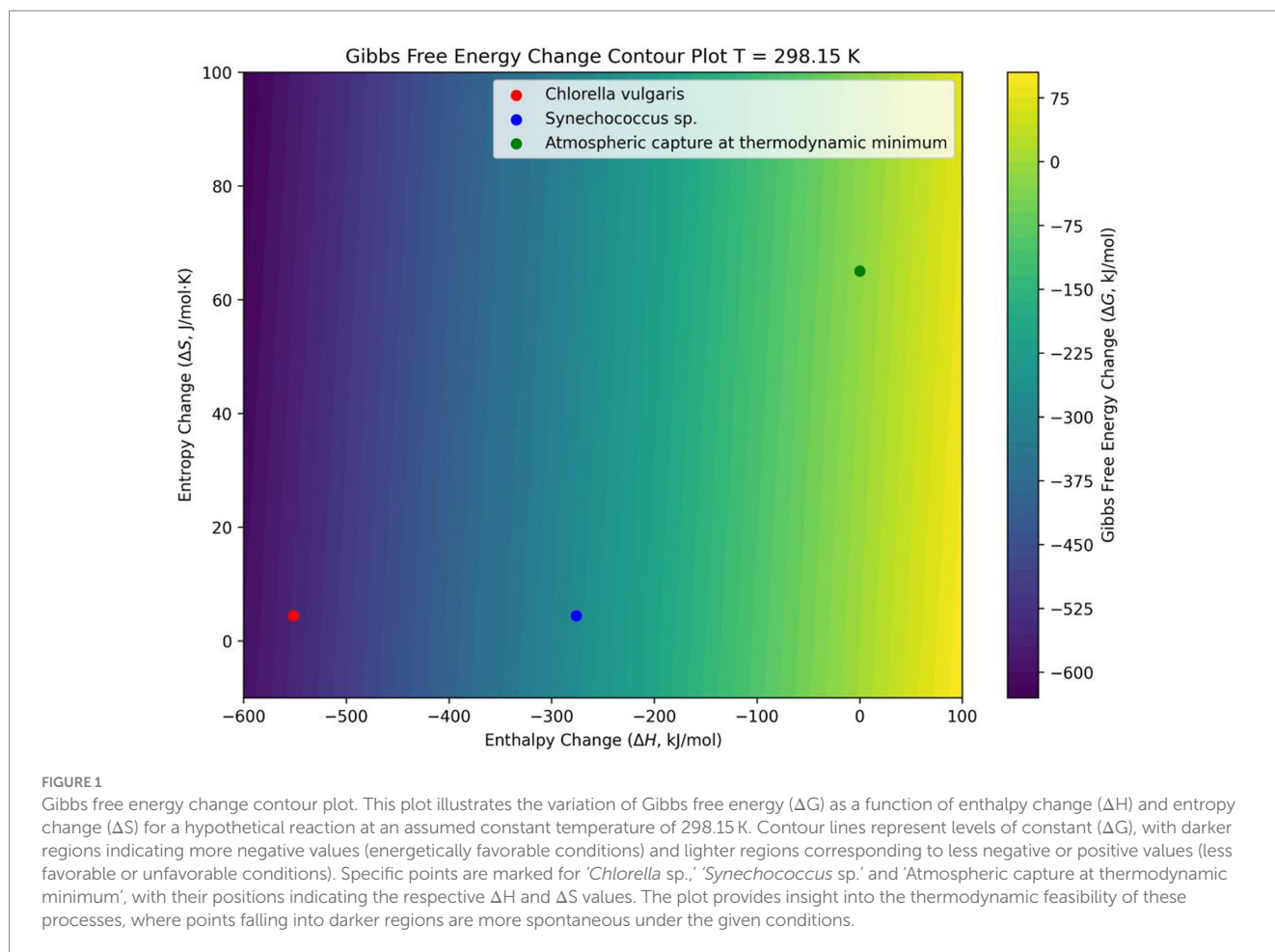
The ΔS° for both reactions 1 and 2 are paradoxically lower per mol of biological CO₂ fixation than that of the thermodynamic minimum for the separation of CO₂ from atmospheric concentrations (Appendix 1.3). It is important to note that, first, this is due to there being two different changes for the state function enthalpy and entropy. Secondly, the thermodynamic analysis focuses on the system changes rather than the universal enthalpy/entropy changes, which can lead to seemingly paradoxical appearances such as a negative

entropy change in a system, when one forgets to consider the increased entropy in the surrounding environment for a living organism. One interesting conclusion from this is that this lower system entropy means that the possibility for improving the process is greater in CO₂ capture using biological processes, through genetic engineering or otherwise, given that the thermodynamic minimum already has a second-law limit that is 14.8 times bigger in a given physicochemical process of separation than that of *Synechococcus* sp. (65.03 vs. 4.4 J/molCO₂.K; Figure 1).

3 Recent discovery of fast-growing species unlocks the possibility of industrial-scale CO₂ fixation using engineered cyanobacteria

The recent discovery of fast-growing cyanobacteria such as *Synechococcus* sp. strains PCC 11901 and PCC 11801, provides possible platforms for further improvement of carbon sinks via genetic modification, utilizing knowledge from previous work in model strains such as *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7002 and *Synechococcus elongatus* PCC 7942 (Table 3). *Synechococcus elongatus* PCC 7942 has been used as a platform for production of polyhydroxyalkanoates (PHA), ethylene, sucrose, aldehydes, free fatty acids and alcohols from CO₂ (Oliver et al., 2013; Dexter et al., 2015; Ungerer et al., 2018b; Santos-Merino et al., 2019). In these species carbon capture at industrial scale was typically a secondary objective. For example, Algenol engineered *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 for production of ethanol in closed, outdoor photobioreactor systems using CO₂ from an emission site (Legere et al., 2017). The discovery of *Synechococcus elongatus* UTEX 2973, which demonstrates fast doubling times at low cell densities of 2.3 h (Yu et al., 2015), sparked interest in isolating fast growing cyanobacteria and transferring genetic toolkits to this new strain, which possesses high sequence homology with *Synechococcus elongatus* PCC 7942 (>99%). The two strains differ by only 53 SNPs, a 7.5 kb deletion and a 188 kb inversion, but *Synechococcus elongatus* UTEX 2973 has a 2.9-fold faster doubling time than *Synechococcus elongatus* PCC 7942 (Yu et al., 2015). Photophysiological studies demonstrated that *Synechococcus elongatus* UTEX 2973 had increased expression of photosystem I (PSI), cytochrome *b_f* and plastocyanin of 1.6, 1.5 and 2.4 fold, respectively (Ungerer et al., 2018a), compared to *Synechococcus elongatus* PCC 7942, which increased electron flux through the thylakoid electron transport chain. Distinct SNPs were identified in the coding region of *rpaA* of *Synechococcus elongatus* UTEX 2973, a known master regulatory gene for the circadian clock. The K134E point mutation in this gene found in *Synechococcus elongatus* UTEX 2973 is not seen in the genomes of other related strains including *Synechococcus* sp. PCC 11901, *Synechococcus elongatus* 7,942 and *Synechococcus* sp. PCC 7002, although the *rpaA* gene itself is well conserved across the genus. While *Synechococcus elongatus* UTEX 2973 has a lysine substitution, both *Synechococcus elongatus* 7,942 and *Synechococcus* sp. PCC 7002 maintain glutamate in this locus. Interestingly, fast-growing *Synechococcus* sp. PCC 11901 also has a point mutation in this exact locus where arginine is substituted instead of glutamate.

Similarly, distinct SNPs in *atpA* and *ppnK* were identified in the same enrichment analysis in this study. Y252C substitution in the



atpA gene changed cysteine in *Synechococcus elongatus* PCC 7942 to tyrosine in *Synechococcus elongatus* UTEX 2973 in the alpha subunit of ATP synthase and it was speculated that this elicits a loss in sensitivity to redox regulation (Wang et al., 2013). The D260E substitution in *ppnK* results in the change of glutamate in *Synechococcus elongatus* PCC 7942 to an aspartate in *Synechococcus elongatus* UTEX 2973. In the encoded NAD⁺ kinase enzyme, structural analysis has revealed that the shorter negative side chain of the aspartate is electrostatically repelled by the phosphate groups in NAD⁺ and ATP. Kinetic parameters of the resulting NAD⁺ kinase confirmed that the V_{\max} improved from 434 to 625 nmol/min/mg for NAD⁺ in *Synechococcus elongatus* PCC 7942 to *Synechococcus elongatus* UTEX 2973 (Ungerer et al., 2018b), demonstrating a major breakthrough in engineering targets discovered through comparative genomics and bioinformatics. When all five SNPs were introduced into *Synechococcus elongatus* PCC 7942 through markerless cloning, the doubling time was improved from 6.8 to 2.2 h and conferred high-light tolerance (Ungerer et al., 2018b). It was also noted that the introduction of the SNP in the *rpaA* promoter and the pair of coding SNPs did not confer any improved growth phenotype when introduced separately. Future comparative genomics studies across the fast-growing strains that have been isolated may identify the determinants of improved photosynthetic yields. However, *Synechococcus elongatus* UTEX 2973 does not show higher biomass accumulation than *Synechocystis sp.* PCC 6803 or *Synechococcus*

elongatus PCC 7942, limiting its use in biotechnology (Włodarczyk et al., 2020; Mills et al., 2022).

Synechococcus sp. PCC 11801 and *Synechococcus sp.* PCC 11802 are closely related fast-growing strains recently isolated in India (Jaiswal et al., 2018, 2020). *Synechococcus sp.* PCC 11801 is a freshwater strain which shares 83% sequence homology with its two most closely related strains, *Synechococcus elongatus* UTEX 2973 and *Synechococcus elongatus* PCC 7942 (Jaiswal et al., 2018). *Synechococcus sp.* PCC 11802 has 97% sequence homology with *Synechococcus sp.* PCC 11801, with 94,540 SNPs shared between the two species. Interestingly, of the five major SNPs found in the *Synechococcus elongatus* UTEX 2973 *atpA*, *ppnK* and *rpaA* genes that conferred faster growth rates, four were also found in *Synechococcus sp.* PCC 11801 and *Synechococcus sp.* PCC 11802 (Jaiswal et al., 2020). It was also shown that *Synechococcus sp.* PCC 11802 has a different CO₂ specificity and grows better at 1% CO₂, unlike *Synechococcus sp.* PCC 11801, which has a fourfold increase in productivity when grown in ambient air compared to 1% CO₂ conditions.

Synechococcus sp. PCC 7002 has a 2.6-h doubling rate under optimal conditions and is naturally competent, making it another strong candidate since its isolation in the 1960s (Baalén, 1962; Batterton and Van Baalen, 1971; Nomura et al., 2006; Ludwig and Bryant, 2012). However, it was not until the 2010s that major interest in engineering this strain began to emerge. *Synechococcus sp.* PCC 7002 achieved proof-of-concept as a euryhaline

TABLE 3 Biomass productivities of the most widely studied cyanobacteria strains.

(A) Lab (<100 mL) flask scale experiments					
Strain	*Maximum productivity (gDCW/L/d)	Maximum density (gDCW/L)	CO ₂ (%)	Light (μmols/m ² /s ⁻¹)	References
<i>Synechococcus elongatus</i> (PCC 7942)	0.4	1.2	5	400	Broddrick et al. (2016); Ungerer et al. (2018b)
<i>Synechococcus elongatus</i> (PCC 7942)	0.63	6.3	1	750	Broddrick et al. (2016); Włodarczyk et al. (2020)
<i>Synechococcus</i> sp. (PCC 7002)	0.93	9.3	1	750	Hendry et al. (2016); Vijayakumar et al. (2020); Włodarczyk et al. (2020)
<i>Synechococcus</i> sp. (PCC 7002)	1.92	19.2	5	750	Hendry et al. (2016); Vijayakumar et al. (2020); Włodarczyk et al. (2020)
<i>Synechococcus elongatus</i> (UTEX 2973)	1.07	3.2	5	900	Hendry et al. (2019); Ungerer et al. (2018b)
<i>Synechococcus elongatus</i> (UTEX 2973)	0.65	6.5	3	500	Hendry et al. (2019); Włodarczyk et al. (2020)
<i>Synechocystis</i> sp. (PCC 6803)	0.69	6.9	1	750	Sarkar et al. (2019); Włodarczyk et al. (2020); Yu et al. (2015)
<i>Synechococcus</i> sp. (PCC 11801)	N/A	N/A	0.04	1,000	Jaiswal et al. (2018, 2023)
<i>Synechococcus</i> sp. (PCC 11802)	0.95	2	1	1,000	Jaiswal et al. (2020, 2023)
<i>Synechococcus</i> sp. (PCC 11901)	1.83	18.3	1	750	Włodarczyk et al. (2020)
<i>Synechococcus</i> sp. (PCC 11901)	2.33	32.6	5	750	Włodarczyk et al. (2020)
<i>Cyanobacterium aponinum</i> (UTEX 3222)	2.59	31.13	1	750	Schubert et al. (2023)

(B) Outdoor trials					
Strain	Location	Cultivation method	Volume (L)	Productivity (gDCW/m ² /d)	References
Cyanobacteria soda-lake consortia	Alberta, Canada	Tubular PBR	1,150	3.1	Haines et al. (2022)
Cyanobacteria soda-lake consortia	Alberta, Canada	Tubular PBR	1,150	5.8	Haines et al. (2022)
<i>Synechococcus</i> sp. (PCC 7002)	Arizona, USA	Raceway pond	1,025	8.8	Davies et al. (2021)
<i>Synechococcus</i> sp. (PCC 7002)	Arizona, USA	Flat panel PBR	110	9.2	Davies et al. (2021)
<i>Synechococcus elongatus</i> (PCC 7942)	India	Flat panel PBR	100	4.91	Sawant et al. (2021)
<i>Synechococcus elongatus</i> (UTEX 2973)	Texas	Cylinder	20	48	Long et al. (2022)

*Where the duration of the growth assay is beyond the point at which a strain has reached its plateau in biomass density, we have taken the earliest timepoint where the maximum biomass density is reached to be the assumed duration to calculate the productivity.

cyanobacterial chassis for the biosynthesis of a range of valuable compounds including mannitol, riboflavin, cellulose and reducing sugars (Zhao et al., 2015; Ruffing et al., 2016; Kachel and Mack, 2020). Under optimal conditions, *Synechococcus* sp. PCC 7002 was shown to be capable of achieving approximately 9.3 gDCW/L after 8 days in optimized 'MAD' media for marine strains, surpassing that of its fast-growing freshwater counterparts, *Synechococcus elongatus*. PCC 7942 and *Synechococcus elongatus* UTEX 2973, which both achieve a maximum of 6.5 gDCW/L in the same time period (Włodarczyk et al., 2020). Moreover, systems for precise unmarked editing of this species have been developed, allowing repeated chromosomal manipulation (Jones et al., 2021).

In 2020, the euryhaline strain, *Synechococcus* sp. PCC 11901, was isolated and shown to significantly surpass the maximum specific growth rate of other cyanobacteria, demonstrating 18.3 gDCW/L in 8 days in 1% CO₂ at 30°C under 750 μmol photons m⁻² s⁻¹ light, nearly double that of the second most productive strain, *Synechococcus* sp. PCC 7002 (Włodarczyk et al., 2020; Table 3). Further work was conducted to optimize the growth conditions, showing that when cultured with 5% CO₂ in enriched media termed 'MAD2 media', *Synechococcus* sp. PCC 11901 and *Synechococcus* sp. PCC 7002 could yield 32.6 and 19.2 gDCW/L, respectively, in 14 days. Carbon accounts for almost half of the dry weight of microalgae biomass, hence approximately 1.83 kg of CO₂ can be fixed in every 1 kg of microalgae biomass production. The significance of this productivity in the context of industrial biotechnology is that 2.29 g/L/d of biomass productivity or approximately 4.2 gCO₂/L/d of CO₂ fixation can theoretically be accomplished with *Synechococcus* sp. PCC 11901 under controlled conditions in laboratory photobioreactors. While productivity losses are to be expected when scaling up in outdoor photobioreactors (Table 3), the robust growth phenotype observed in fast-growing strains may confer an advantage in scale-up translatability when compared against similar strains of microalgae deployed in commercial photobioreactors (Cheah et al., 2015; de Vree et al., 2015; Ullah et al., 2015). To improve *Synechococcus* sp. PCC 11901 as a chassis for industrial production, further studies characterized the neutral sites for stable genomic integration and also tested a suite of strong promoters for constitutive expression alongside development of a dCas9-based CRISPR interference system for markerless chromosomal genetic modification using CRISPR-Cas12a, thus opening up significant possibilities of using this species as a photoautotrophic workhorse for synthetic biology (Victoria et al., 2023).

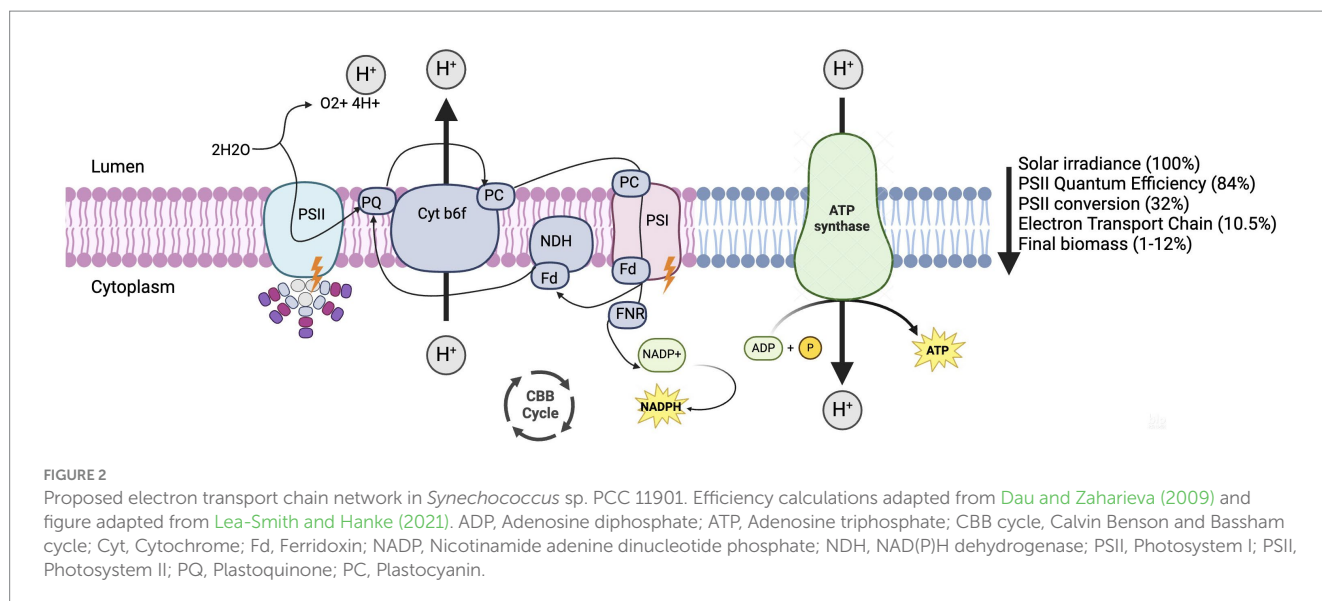
Recently, a novel cyanobacterium, *Cyanobacterium aponinum* UTEX 3222, was identified from a unique marine environment with naturally elevated CO₂ (>98%) and low pH (<6.5; Schubert et al., 2023). *Cyanobacterium aponinum* UTEX 3222 was shown to outpace most other fast-growing strains with a doubling rate of 2.35 h in liquid medium and a biomass density of >31 gDCW/L in batch culture. In addition, *Cyanobacterium aponinum* UTEX 3222 was shown to sink more quickly than other fast-growing strains, which was attributed to its larger cell volume (Schubert et al., 2023). This property is extremely useful for industrial-scale cultivation as it reduces the requirement of industrial flocculants or dewatering. However, tools to genetically manipulate this species have not been developed.

4 Using carbon sinks for enhanced CO₂ fixation

4.1 Rationale behind carbon sinks as a method of driving CO₂ fixation

Electron transport in cyanobacteria has been recently reviewed so we will focus on the energy conversion rates (Lea-Smith and Hanke, 2021). Moreover, there is some variation in the electron transport chain between cyanobacteria, so for simplicity, the predicted pathway in *Synechococcus* sp. PCC 11901 is shown in Figure 2 (Lea-Smith et al., 2013; Mills et al., 2022). The first step is driven by photosystem II (PSII), where water is split by sunlight into oxygen, protons, and electrons, which replaces energized electrons released from the reaction center. The quantum efficiency of this process is 84% (Wientjes et al., 2013), although the transfer of useful work from the light harvesting complex (LHC) to the water splitting reaction is around 32%, as shown in Figure 2 (Dau and Zaharieva, 2009). Ultimate transfer of the electron to ferredoxin-NADP⁺-reductase to reduce NADP⁺ to NADPH and production of a proton gradient across the thylakoid membrane, which drives ATP production via ATP Synthase, results in a direct conversion of light energy into chemical potential energy that is usable by the cell (Dau and Zaharieva, 2009). Dau and colleagues determined that the energy efficiency of this first stage is 10.5% (Dau and Zaharieva, 2009). However, between the light dependent reactions and the Calvin-Benson-Bassam (CBB) cycle, there is a significant loss in energy transfer, resulting in only 1–2% of energy converting to biomass, although this might be higher in certain cyanobacteria and algae, potentially up to 10%, which is close to the theoretical maximum of 12% (Ooms et al., 2016; Hann et al., 2022). During the process of carbon fixation, NADPH and ATP are utilized in the Calvin-Benson-Bassham (CBB) cycle to regenerate ribulose 1,5-bisphosphate, which Ribulose bisphosphate carboxylase/oxygenase (rubisco) catalyzes the addition of CO₂ (CO₂ fixation) to in order to form two molecules of 3-phosphoglycerate, that subsequently enter central metabolism. Rubisco can also catalyze the addition of O₂ to ribulose 1,5-bisphosphate, resulting in production of 2-phosphoglycolate, a process called photorespiration, which reduces carbon fixation rates. It has been widely accepted that the CBB cycle in photosynthetic organism is bottlenecked by the slow reaction kinetics of rubisco which fixes only 1–22 CO₂ molecules per second per active site (Zhao et al., 2024), in part due to the requirement for specificity of CO₂ over O₂ binding. However, carboxylation rates are faster in cyanobacterial species, which is likely linked to the lower requirement for CO₂ specificity, as rubisco is enclosed in the CO₂ enriched carboxysome (Savir et al., 2010). Attempts to structurally engineer rubisco have rarely shown worthwhile improvements in carbon fixation rates, including in cyanobacteria (Oliver and Atsumi, 2015), and it has been suggested that despite its slow catalysis, it is already perfectly optimized to accommodate the environmental CO₂ concentration (Tcherkez et al., 2006).

A significant amount of harvested light energy can be dissipated in deleterious reactions under low CO₂ conditions because of the slower reaction kinetics in the CBB cycle, leading to insufficient regeneration of NADP⁺ and ADP, which in turn results in over-reduction of the electron transport chain (Kanno et al., 2017). As a result, during times of excessive light intensity, instead of capitalizing on this energy to produce more biomass, most of this energy is



transferred to O_2 via either photorespiration, controlled reactions in terminal oxidases or flavodiiron complexes, or via uncontrolled reactions resulting in formation of reactive oxygen species (ROS), which induces cell stress and photo-oxidative damage, ultimately leading to cellular death (Santana-Sanchez et al., 2019; Lea-Smith and Hanke, 2021). A (re-)balance between light harvesting and energy usage/storage by the cell is needed to optimize the overall light energy conversion efficiency.

The slowness of the reaction kinetics downstream of the CBB cycle creates a metabolic bottleneck (Ducat et al., 2012; Hendry et al., 2016). Several of the metabolic pathways are heavily regulated by proteins sensitive to the cytoplasmic redox state that is used as a proxy for detecting the energetic 'saturation' of the cell. These mechanisms, such as *cp12*, downregulate the upstream pathways involving photosynthesis under these conditions. Rational engineering to redirect carbon partitioning toward less bottlenecked pathways or inserting heterologous enzymes may enable the synthesis of *de novo* carbon-dense compounds and relieve upstream CO_2 fixing steps from inhibitory feedback loops, potentially maintaining a higher rate of carbon fixation for a longer period of time (Oliver and Atsumi, 2015). This is confirmed through experimental findings but is also in line with the conclusions drawn from the thermodynamic calculations concerning the second-law limits of *Synechococcus* sp. biomass discussed at the end of Section 1.

4.2 Rational engineering of carbon sinks into metabolic pathways

While photosynthesis is energetically more efficient than physicochemical means of CO_2 capture, it faces limitations including inefficient light absorption due to pigment selectivity, temperature sensitivity affecting enzyme activity, constraints in CO_2 and water availability, photorespiration and CBB cycle inefficiency due to ATP and NADPH availability. Genetic improvements in cyanobacteria have focused on enhancing photosynthetic efficiency, including overexpressing rubisco (Jensen, 2000; Liang and Lindblad, 2017; Tharasirivat and Jantaro, 2023), although this may be limited by CO_2

availability and the inability to pack additional rubisco into carboxysomes. Therefore, a more logical approach involves modulating the carbon flux to optimize carbon sinks, ensuring efficient utilization of fixed carbon for biomass production and metabolic processes. This strategy complements efforts to improve carbon fixation by addressing downstream reactions, ultimately maximizing the overall efficiency of photosynthesis and biomass accumulation in cyanobacteria. Redirecting fixed carbon toward the synthesis of C dense sinks not only aids in carbon sequestration but also fosters sustainable production of valuable bioproducts (Oliver and Atsumi, 2015). For this, targeting genes linked to biosynthesis of sugars, glycogen, alkanes, fatty acids, esters etc., has been identified as a strategic approach to optimize C sinks for improved carbon sequestration and bioproduct yields.

In *Synechococcus elongatus* PCC 7942, heterologous expression of *cscB*, encoding a sucrose symporter, enabled cytosolic sucrose to be rapidly exported into the media, but also increased biomass productivity and photosynthetic activity (Ducat et al., 2012). Increased flux toward sucrose production was achieved by knocking out *glgA*, encoding a protein required for synthesis of the alternate glycogen carbon sink, and *invA*, encoding a protein which breaks down sucrose to glucose and fructose (Figure 3). In an alternate experiment, three genes encoding acetolactate synthase (AlsS), 2-acetolactate decarboxylase (AlsD), and secondary alcohol dehydrogenase (Adh) were expressed to enable production of 2,3-butanediol (2,3-BD), achieving production of 2.4 g/L in 21 days (Figure 3; Oliver et al., 2013). Since pyruvate is a precursor to multiple anabolic pathways and to the 2,3-BD pathway, it is reasonable to expect that increasing the pool of pyruvate would increase the availability for 2,3-BD synthesis as a carbon sink. By overexpressing *pgm* and *pyk* encoding phosphoglyceromutase and pyruvate kinase respectively, resulting in higher production of pyruvate, the recombinant strains had slight attenuated biomass production but an improved overall carbon and 2,3-butanediol yield of 1.8 and 2.4-fold, respectively (Figure 3; Oliver and Atsumi, 2015). This demonstrates that introducing certain carbon sinks can increase CO_2 fixation rates. However, increasing production of free fatty acids to 6.16 mM (1.54 g/L) by knocking out the long-chain-fatty-acid-CoA ligase *fadD* and inserting a truncated

overexpressed in a mutant strain of *Synechococcus elongatus* PCC 7942 engineered to produce 2,3-BD (Figure 3). This combination of CBB cycle disinhibition in a strain resulted in 39% higher production of 2,3-BD from CO₂ (Kanno et al., 2017).

In studies conducted in *Synechococcus elongatus* PCC 7942, 1,3-propanediol (1,3-PDO) production of 1,3-PDO was improved by optimization of gene expression and growth conditions. First, a synthetic pathway consisting of genes derived from *Escherichia coli*, *Saccharomyces cerevisiae* and *Klebsiella pneumoniae* were introduced to produce 1,3-PDO directly from CO₂. The strain produced 0.29 g/L 1,3-PDO, however, it also accumulated 1.32 g/L glycerol as an intermediate product (Hirokawa et al., 2016). To reduce glycerol accumulation and improve 1,3-PDO production, the weaker promoter, P_{LlacO1}, was replaced with a stronger promoter (P_{trc}), and induction timing for gene expression and medium composition were optimized. This resulted in higher 1,3-PDO production (1.22 g/L) and reduced glycerol accumulation (0.87 g/L; Hirokawa et al., 2017a).

4.3 Metabolic flux balance analysis and CRISPR-interference pooled screening identifies novel gene targets

Earlier generations of metabolic models in cyanobacteria were developed considering the subsystems in isolation, such as the circadian clock (Miyoshi et al., 2007), light dependent reactions (Jablonsky and Lazar, 2008) and the CBB cycle (Badger et al., 1985), have been useful in elucidating reaction kinetics. Flux balance analysis (FBA) enables a comprehensive reconstruction of cellular metabolism and has been used to test the phenotypic effects of genetic perturbations (Orth et al., 2010). However, this is significantly harder to perform in a photoautotrophic organism with an internal circadian rhythm because of the additional dynamic parameters introduced, compared to well-characterized heterotrophs such as *Escherichia coli* (Reimers et al., 2016). Since FBA assumes a steady-state, introducing a temporal component to the model where light intensity varies will overcome the major limitations in the earlier genome-scale metabolic modeling of photoautotrophic organisms, as has been demonstrated for *Synechococcus* species (Vijayakumar and Angione, 2017; Sarkar et al., 2019; Vijayakumar et al., 2020). Combined with multi-omics data and computational approaches, dynamic FBA is a powerful tool capable of elucidating novel genetic targets and quantifying the predicted change in carbon partitioning. For example, the synthetic metabolic pathway successfully tested by Hirokawa et al. (2016) for 1,3-PDO production was incorporated into the genome-scale model of *Synechococcus elongatus* PCC 7942 for FBA. This FBA model allowed the authors to identify novel NADPH dehydrogenase 1 (NDH-1) complexes and disruption of the gene encoding the NdhF1 subunit of NDH-1, resulting in production of 0.34 g/L 1,3-PDO and 2.79 g/L glycerol (Hirokawa et al., 2017b).

Though FBA has been developed for *Synechocystis* sp. PCC 6803, *Synechococcus elongatus* UTEX 2973, *Synechococcus* sp. PCC 7002 and *Synechococcus elongatus* PCC 7942, transcriptomics and metabolomics data for the fastest growing strain, *Synechococcus* sp. PCC 11901, are not yet available and FBA has not been modeled (Table 2). Furthermore, performing FBA in scaled up outdoor cultivation conditions under diurnal light cycles will be of significant value to

industry and may implicate a different set of genetic targets for a more efficient carbon sink target.

4.4 Improving carbon flux through bicarbonate transporters

Cyanobacteria concentrate dissolved inorganic carbon (DIC) species inside the cell at up to 1,000-fold the concentration of the surrounding environment, due to its unique CO₂ concentrating mechanisms (CCM; Badger and Price, 2003). Central to the CCM is the carboxysome which contains rubisco and carbonic anhydrase, which converts HCO₃⁻ into CO₂ and improves the overall reaction kinetics by increasing substrate and enzyme concentrations. It has previously been shown that there are several modes of DIC uptake in cyanobacteria. Two of the four NDH-1 complexes found in the thylakoid membrane, NDH1-MS and NDH1-MS', are involved (Price et al., 2013; Hualing, 2022). Cyanobacteria also express plasma membrane bound bicarbonate transporters with different binding affinities to CO₂ and bicarbonate ions: BCT1 which is a medium affinity ATP-binding cassette transporter; SbtA, an inducible sodium ion-dependent, low flux bicarbonate transporter; and BicA, a constitutively expressed sodium ion-dependent high flux transporter (Gupta et al., 2020). Transcriptomic studies revealed increased expression of *bicA* and *sbtA* under carbon limitation. Overexpression of *sbtA* and *bicA* under strong inducible promoters in *Synechococcus* sp. PCC 7002 was shown to increase biomass dry cell weight yields by 50% compared to wild-type (Gupta et al., 2020). These mutations, in combination with an engineered carbon sink, may be synergistic in developing improved strains for carbon sequestration.

4.5 CRISPR-interference pooled screening of carbon sink expressing mutants

Yao et al. pioneered the use of pooled CRISPR interference libraries for targeted gene repression to screen genes in cyanobacteria (Yao et al., 2020). With this method, a pool of 10,498 clones of *Synechocystis* sp. PCC 6803 were generated, each expressing dCas9 and an sgRNA that targeted a unique protein-coding open reading frame or a non-coding RNA. This enabled a fitness score to be ascribed to each of these genes after screening the mutant pool under multiple growth conditions and performing enrichment analysis post-next generation sequencing (NGS).

Building on this, Li et al. demonstrated the use of CRISPRi in *Synechococcus elongatus* PCC 7942, specifically in a CRISPR-engineered mutant expressing heterologous *alsS*, *alsD* and *adh* to rewire carbon flux from pyruvate to 2,3-BD synthesis as a carbon sink (Li et al., 2022). Four enzymes were identified which catalyzed reactions that directly competed with the partitioning of carbon toward pyruvate from the CBB cycle. Fructose biphosphatase (Fbp) and sucrose phosphate (SpsA) convert fructose-1,6-bisphosphate (FBP) to fructose-6-phosphate (F6P) and then F6P to sucrose, respectively, thus drawing carbon out of the CBB cycle. Phosphoenolpyruvate (PEP) is produced from 3-phosphoglycerate (3PG) that directly exits the CBB cycle and is a precursor to pyruvate. PEP is also converted into oxaloacetate (OAA) by the PEP carboxylase (Ppc) enzyme which directly competes with the flux of PEP to

pyruvate. Lastly, pyruvate is also converted into acetyl-CoA by pyruvate dehydrogenase (Pdh; Figure 3). Therefore, it was speculated that repression of these genes with CRISPRi may improve 2,3-BD yields as a carbon sink. By inserting sgRNA cassettes that target these four genes, they were knocked down both independently and in combination. The multiplex suppression of all four genes resulted in 2,3-BD titers increasing from 792.8 mg/L to 1,583.8 mg/L, without incurring any biomass growth inhibition. CRISPRi pooled libraries may be useful for elucidating the genetic basis of improved CO₂ fixation rates in newly discovered fast growing strains such as *Synechococcus* sp. PCC 11901 and may also serve as a powerful tool for improving fast-growing strains to produce carbon sinks by enhancing carbon flux toward desirable pathways by knocking down/out competing enzymes in a combinatoric approach.

4.6 Challenges and future perspectives

It has been suggested that microalgal BECCS at 15 g/m²/d productivity achieves a comparable cost and electrical requirement but a significantly lower heating requirement than current DACCS technologies (Even et al., 2022). A recent smaller scale, continuous cultivation, outdoor trial with *Synechococcus elongatus* UTEX 2973 engineered to express limonene synthase, demonstrated productivities of up to 48 g/m²/d (Long et al., 2022). This strain, however, has not been tested in larger scale photobioreactors (Table 3). Improved biomass accumulating strains such as *Synechococcus* sp. PCC 11901 may achieve even higher yields but, to the best of our knowledge, have not been tested in larger photobioreactors outdoors. Bridging this translational gap from bench-to-pilot scale remains a challenge for deploying microorganisms for atmospheric carbon removal or BECCS (Khan et al., 2019). This may be of increasing interest as a solution to achieving gigatonne scale decarbonization if global energy availability becomes a constraint. Focusing on carbon sinks is also a potential strategy for channeling carbon flux toward more appropriate biosynthesis pathways. However, the majority of this work has been performed through rational design, and there has only been limited evidence in the literature of where the chosen targets were guided precisely by advanced screening techniques such as CRISPRi or multi-omics data. Metabolic engineering through targeted genetic manipulation presents a promising avenue to enhance carbon fixation rates and adaptability to flue gases, while simultaneously offering routes to diversify revenue streams, thereby improving commercial viability (Satta et al., 2023). However, the relatively limited repertoire of sophisticated genetic regulatory components tailored for precise control of introduced pathways in cyanobacteria renders this field years behind biomanufacturing industries using more well-established organisms like *E. coli* and *Saccharomyces cerevisiae* (Jones, 2014; Hitchcock et al., 2020; Guillaume and Branco dos Santos, 2023). Recent efforts have focused on characterizing standardized modular cloning parts in cyanobacteria, such as the CyanoGate system, yet the advancement of such parts could be significantly boosted by establishing robust and modular expression libraries (Vasudevan et al., 2019; Caicedo-Burbano et al., 2020). Furthermore, expanding genomic, expression, and mutant libraries may provide deeper insights into cyanobacterial physiology, facilitating metabolic engineering efforts.

While CRISPR-based genome editing and interference approaches have shown promise in regulating gene expression by targeting multiple loci simultaneously, concerns regarding toxicity and off-target effects of CRISPR nucleases may limit their application in cyanobacterial genome modification (Guo et al., 2023). Efforts to mitigate this issue include controlling the expression of CRISPR nucleases post-introduction into cyanobacteria (Mengstie et al., 2024). Despite these challenges, the ongoing development of CRISPR-Cas systems in cyanobacteria holds tremendous potential for strain engineering. Future research in synthetic biology aimed at optimizing cyanobacteria as a chassis for metabolic carbon sinks should benefit significantly from the proven approaches using multi-omics guided flux balance models, and high-throughput screening methods. Additionally, the establishment of scalable cultivation systems and outdoor testing platforms is crucial for validating engineered strains under real-world conditions and also gaining a better understanding of how flux balance changes according to diurnal light cycles—for which there is still limited understanding in the field.

Performing carbon removal through photosynthesis has long been compared against competing chemical technologies. It must be noted that drawing an energetic comparison against non-biological systems is difficult, since biology is unable to uncouple its entropy and enthalpy changing activities. A cell performs three combined functions analogous to a PV panel (thylakoid), a direct air capture unit (Ci-transporters) and a CO₂ upcycling system (CBB-cycle), that cannot be separated. Strictly speaking, since the separation of CO₂ from the air requires only an entropic change, from the perspective of maximizing just CO₂ separation, biological systems that perform additional work to undergo an enthalpy change is unnecessary or wasteful. However, from the more pragmatic perspective, we make the argument that the inevitable enthalpy change undergone by CO₂ in photosynthesis ought not to be considered as an inefficiency, but rather, a mechanism by which carbon removal at the 10 Gt/year scale can become net profitable instead of being a permanent cost to the planet. For any carbon removal method that does not create an enthalpy change, CO₂ separation will always be at a net cost if captured CO₂ is being buried, with some models projecting costs upward of \$3 trillion/year at the 10 Gt/year scale (Sievert et al., 2024). A CO₂ drawdown of 10 Gt/year is equivalent to 2.7 Gt of carbon atoms by mass fraction, which to put into perspective, is equivalent to 63% of the mass of carbon atoms contained in all the crude oil that the world produces each year (4.3 Gt; IEA, 2023). Therefore, the chemical potential energy that biological systems can efficiently absorb from sunlight to transform carbon opens up possibilities for a new era of carbon-negative industrial manufacturing that can offset the costs of co-performing carbon sequestration as biomass—which may be a more sustainable way to continuously conduct 10Gt/y of carbon removal (Cestellos-Blanco et al., 2020; Stephens et al., 2021).

Without taking into account energy gained in enthalpy change for a given area of cyanobacterial cultivation, the overall mass of CO₂ sequestered and converted into biomass per square meter of solar irradiance is significantly less than the mass of CO₂ that would be separated from the air by employing a given atmospheric carbon removal technology using photovoltaics. Assuming that a PV panel can power a given atmospheric carbon removal method that uses 1,250 kWh/T CO₂ to perform separation, if the energy is produced from a m² of photovoltaic panels, assuming that there is a 100% energy transfer efficiency between the PV panel and carbon removal

technology, we can draw a direct comparison against the areal efficiency of biological CO₂ sequestration based solely on its areal biomass productivity. Taking the solar irradiance data for the month of September in Arizona to draw this comparison, we estimate that a typical PV panel receives 6.8 kWh/m²/day of light energy and is capable of producing 0.8 kWh/m²/day of electrical energy, assuming a standard 16% overall efficiency, 14.08% system losses, 96% inverter efficiency and 20.2% DC capacity factor (Abdelhamid et al., 2016). Taking this further, assuming that atmospheric carbon removal can be done at 1,250 kWh/T CO₂, approximately 640 g/d of CO₂ can be captured for each m² of additional PV capacity built for powering carbon removal based on these assumptions. In one recent techno-economic model at a similar latitude in Nevada, it was proposed that a 100 MW capacity of PV and 300 MWh battery would be required to remove 100,000 T CO₂/year using direct air capture and storage powered by solar energy (DACCS). Assuming that 1 m² of solar PV produces 0.16 kW of electricity, a 100 MW capacity PV system would equate to 625,000 m² of required capacity removal or 438 g CO₂/day at a levelized cost of \$877/T CO₂, assuming a carbon removal efficiency of 88% (NREL PVWatts Calculator, 2014; Gutsch and Leker, 2024). Comparing this to cyanobacterial cultivation, 43.3 g/m²/d of biomass yield was achieved using an outdoor pond-design photobioreactor with *Synechococcus elongatus* UTEX 2973 in September 2021 in Texas at a similar latitude, an equivalent CO₂ fixation of 81 g/m²/d, assuming a biomass to CO₂ stoichiometric ratio of 1:1.88 (Long et al., 2022). Outdoor algal cultivation trials in Arizona by NREL at a similar latitude showed 19.9 g/m²/d productivity in September with the microalgal strain *Picochlorum celeri* (Klein and Davis, 2023). Therefore, it can be assumed that from the perspective of areal efficiency of converting incident solar energy into CO₂ removal, PV panels convert enough solar energy to deliver 5–8 times the amount of CO₂ removal at 1,250 kWh/TCO₂ than using *Synechococcus elongatus* UTEX 2973 at this productivity rate. Therefore, biomass productivities need to be improved if biological carbon sequestration is to reach parity with PV-powered atmospheric carbon removal for a given area of incident solar energy.

Missing from the above calculation is a consideration of the enthalpy change that is recoverable against the irrecoverable work done against entropy in both of those examples (Table 2). *Synechococcus* sp. have an advantage in that there is an additional gain of 274 kJ/mol CO₂ fixed as biomass as an enthalpy change (1,729 kWh/T CO₂ in chemical potential energy gained), which is theoretically recoverable as work, unlike work done against entropy. It is also interesting that the work done against system entropy is significantly lower in biological solutions that sequester CO₂ than the minimum work done to perform CO₂ separation. Therefore, a strong argument exists based on thermodynamics to use engineered bacteria to produce carbon sinks as a method of deriving chemical (and economic) value from this enthalpy change instead of the burial of carbon, even if only partially. Secondly, even if we were to, for the sake of argument, nullify the energetic gain as a sunk cost, the cost of deploying a fully PV powered DAC infrastructure imposes a levelized cost of capture of \$877/T CO₂ based on recent models (Gutsch and Leker, 2024). Conversely, an algal system amounts to \$320/T CO₂ (calculated from the minimum biomass selling price of \$602/T biomass) based on NREL's techno-economic analysis which assumes a modest average of 18.5 g/m²/d of annual productivity (Klein and Davis, 2023), before any consideration of possible additional revenue routes from engineered

carbon sinks and bioproducts. Therefore, gigatonne-scale carbon removal will be economically burdensome irrespective of technology, unless further value can be generated from the captured CO₂, which fundamentally requires an enthalpy increase. If this route is pursued, from a thermodynamic perspective, it would be reasonable to speculate that this may be significantly more favorable to perform biochemically than by stacking atmospheric CO₂ separation with a chemical reaction converting CO₂ into precursor compounds, which would be illustrated in the comparison of the total Gibbs free energy change as aforementioned. However, further work is required to harness light-driven biosynthesis and there is considerable process scale-up challenges, as well as legislative challenges concerning the use of modified organisms that need to first be overcome before this technology can become viable.

5 Conclusion

CDR through harnessing cyanobacteria for CO₂ fixation presents promising avenues for mitigating the impacts of climate change. At the crux of the issue, the thermodynamics underlying atmospheric carbon removal presents a major problem concerning competing renewable energy resources as well as an economic burden when imagined at the 10 Gt/year scale. Using photosynthesis has limitations such as the surface area required, although it has the advantage of being self-powered, rendering it energetically additional and independent of renewable energy sourcing constraints. Furthermore, since it undergoes both an entropic and enthalpy change, it presents a possibility of becoming a net profitable operation if some economic value can be derived from the additional work done to convert CO₂ into higher energy state compounds by the cell. The genetic engineering of carbon sink pathways unlocks a new route by which CO₂ removed from the atmosphere can be converted into value while carbon partitioned toward cell growth is locked for permanent carbon removal. The carbon sinks in the evidence discussed in this review shows that biosynthesis does not necessarily impede overall CO₂ fixation rates, but rather can increase it further if the metabolic flux is carefully considered. Further multi-disciplinary research on using fast-growing cyanobacteria, such as *Synechococcus* sp. PCC 11901, may lead to commercially viable, industrial-scale CO₂ fixation. Genetic modification and advanced multi-omics analyses have led to significant enhancements in carbon sink capacities and overall CO₂ fixation rates. Integration of metabolic FBA and CRISPRi pooled screening facilitates the identification of novel gene targets crucial for further improving CO₂ fixation efficiency in cyanobacteria. With continued research efforts focusing on key metabolic pathways, genetic engineering strategies, and industrial-scale cultivation techniques, the prospect of performing carbon removal that circumvents many of the major foreseeable challenges concerning energy and thermodynamic limitations with currently proposed non-biological methods.

Author contributions

DK: Conceptualization, Investigation, Visualization, Formal Analysis, Writing – original draft. JM-C: Investigation, Writing – original draft. ES: Investigation, Visualization, Formal Analysis,

Writing – original draft. DJL-S: Visualization, Writing – original draft, Writing – review & editing. US: Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

DK, JM-C, ES and US are affiliated with CyanoCapture Ltd.—a biotechnology company conducting research into cyanobacterial physiology and genetics.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fclim.2024.1412232/full#supplementary-material>

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