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Emerging platforms for co-utilization of one-carbon substrates by photosynthetic organisms

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One-carbon substrates have generated increasing attention as long-term sustainable feedstocks for biobased production of fuels and chemicals. However, their physicochemical properties present significant biological and operational challenges for commercial bioprocesses including kinetically slower substrate activation, high energetic cost of assimilation, low mass transfer, substrate toxicity, and low productivity titers. Several different routes including optimization of native pathways, synthetic pathways, and hybrid methods are being explored to overcome these challenges. Integration of emerging biological solutions with process improvements is enabling faster bioprocess development for cost-effective conversion of one-carbon substrates into fuels and chemicals.

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Introduction

Current commercial processes for the bioproduction of fuels and chemicals compete with food and feed for plant-derived carbohydrates [1,2]. A combination of several factors including increasing population (forecasted to be 9.7 billion by 2050), loss of arable land due to urbanization, and impact of climate change on crop productivity have led to a persistent debate about long-term sustainability of carbohydrates as feedstock [3,4]. Furthermore, cost of substrate is a major contributor to the production cost [2]. Therefore, higher feedstock prices could have a significant impact on economic competitiveness of carbohydrate-based bioprocesses. One-carbon (C1) substrates including methane, methanol, formate, carbon monoxide (CO) and carbon dioxide (CO_2) have generated significant interest as long-term sustainable feedstocks among the scientific community and other stakeholders [5•,6–12,13•,14,15]. Importantly, because CO_2 and methane are two major greenhouse gases (GHG), such bioprocesses will help reduce the GHG emission while meeting the societal needs for energy and chemicals.

Progress in understanding of the complex biology of C1 utilizing organisms, synthetic biology tools, and process optimization is enabling expedited development of C1 feedstocks-based bioprocesses [7–9,14–16]. However, fundamental challenges in biological utilization of these feedstocks at scales and rates needed for commercial production remain. This is due to the high energetic cost of substrate assimilation (CO, CH_2O_2, and CO_2), thermodynamic constraints (CO_2 and CH_4), toxicity to production host (CO, CH_2O, and CH_2O_2), and low mass transfer rate (CH_4 and CO) [5•,6,7,10,14]. Therefore, kinetically and energetically efficient biological activation and assimilation of C1 substrates into the central carbon metabolism remains a key focus of research and development [17,18,19••,20]. Multiple native pathways for activation and assimilation of these substrates have been characterized [21–26]. In addition, the availability of an increasing number of genomic sequences should allow data mining for kinetically and energetically better enzymes and pathways to improve efficiency of activation and assimilation of C1 substrates [27]. We highlight recent progress that could potentially help overcome the economic challenges associated with C1 substrates based bioprocesses. Syngas (CO, CO_2, and H_2) fermentation-based bioprocesses have been widely reviewed [7,8,12] and will not be discussed further. We discuss recent advances in natural and synthetic pathways for carbon assimilation by fixation and reduction, with a special focus on oxygenic photosynthetic organisms which have the inherent capability to harvest electrons from sunlight. We also review recent efforts on the development of the photomixotrophic platforms for co-utilization of CO_2, methane, methanol and formate.

CO_2 fixation: natural pathways

Out of the six natural pathways for CO_2 fixation, the Calvin–Benson–Bassham (CBB) cycle, the 3-hydroxypropionate (HP) cycle, and the hydroxypropionate/hydroxybutyrate (HP/HB) cycle are oxygen tolerant [21–26]. Phototrophs such as plants, algae, and cyanobacteria utilize the CBB cycle, the most dominant pathway, to fix >300 gigatons CO_2 annually [19••].

Photosynthetic microorganisms (microalgae and cyanobacteria) have drawn significant interest for their ability to directly convert CO_2 into fuels and chemicals. These organisms grow fast (some can grow as fast as yeast under controlled conditions [28••]) and have higher photoconversion efficiency (PCE) (typically <2%; theoretical maximum ~10% [29]) compared to higher plants (typically <1%; theoretical maximum ~6%) [30]. These attributes could in principle provide an economical path for the direct conversion of CO_2 into products as opposed to a two-step bioconversion of CO_2 into products by heterotrophs using plant-derived carbohydrates. However, downstream processing also plays a significant role in the economic competitiveness of bioprocesses. Heterotrophic organisms produce the targeted product at >1 g/L/h rates and titers can reach to >100 g/L, thus reducing the significant cost associated with operations related to fermentation and extraction of product from broth. A better physiological understanding along with the development of synthetic biology tools should allow engineering of photosynthetic organisms for improved product tolerance, and therefore, high titers. However, the rate of product formation remains a significant hindrance to the development of photosynthetic organism based bioprocesses. Current reported product rates under photoautotrophic conditions are still in the mg/L/h range [13•,14].

The inherent catalytic inefficiency of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (RuBisCO) and the energetic inefficiency of the CBB cycle are two key factors in a low rate of product formation [17,18,19••]. Box 1 shows a hypothetical scenario of ethanol production by a cyanobacterial-based bioprocess. A simple calculation assuming a biomass concentration of 2 g cell dry weight (CDW)/L in a photobioreactor shows that the amount of RuBisCO needed to achieve ethanol production at a rate of 1 g/L/h must constitute 10.8% of the total cellular protein. Furthermore, the energetic requirement for the CBB cycle to sustain this rate of ethanol production requires an input of at least 0.528 mol photon/L/h, which is significantly higher than the capture of solar energy by photosynthetic organisms (0.15 mol photon/m^2/h). It is clear, therefore, that improving the kinetic and energetic efficiencies of CO_2 fixation will increase the rate of product formation, and expedite the development of photosynthetic bioprocesses. Additionally, the improved efficiency of the CBB cycle will reduce the cellular resources associated with protein biosynthesis, enzyme maintenance, and turnover costs.

Box 1 Amount of RuBisCO and solar energy required for the production of ethanol using a cyanobacterium

Amount of RuBisCO	
Proposed rate of ethanol production	1 g/L/h
Required rate of CO_2 fixation	2.9 g/L/h
%RuBisCO/total cellular protein	10.8%[a]
Amount of solar energy	
Number of photons required for CO_2 fixation	0.528 mol/L/h[b]
Photons captured by a cyanobacterium	0.15 mol/m^2/h[c]

[a]RuBisCO turnover rate used is 11.6/s/monomer [60] and monomer mol wt is 70 000 g/mol. Assumed biomass concentration is 2 g DCW/L and total biomass consists of 51% total protein [61].
[b]A minimum of 8 mol photons is required to fix one mol CO_2 [62].
[c]Based on full-spectrum solar energy 7349 MJ/m^2/yr [62], 2% PCE and photon energy 225.3 kJ/mol. Assuming production system operates for 365 days and receives 12 h of solar exposure on a daily basis.

Improving catalytic efficiency of RuBisCO

The ongoing efforts to generate a kinetically efficient RuBisCO have met with little success due to the apparent mutual inverse relationship between the carboxylation efficiency and substrate specificity [31]. Indeed, naturally evolved, kinetically efficient RuBisCOs have poor substrate selectivity between CO_2 and O_2 resulting in a loss of ~25% fixed carbon [32]. However, photosynthetic microorganisms sequester RuBisCO in a specialized microcompartment to shield it from O_2 [33]. Therefore, it can be argued that the improvement in carboxylation efficiency of RuBisCO even at the risk of loss of substrate specificity should be a useful outcome for the photosynthetic bioprocesses. Few *E. coli* based heterologous systems have been developed towards the goal of improving RuBisCO carboxylation efficiency [34,35•,36••]. In one such attempt, *E. coli* was engineered to produce RuBP by expression of the phosphoribulokinase (PRK) [34]. Because RuBP accumulation is toxic to *E. coli*, growth of the engineered strain can be linked to RuBisCO activity. This conditional screen was used to identify mutations that led to the biogenesis of RuBisCO as well as improved the carboxylation efficiency [34]. A drawback of this selection system is the large number of false positives arising from the loss of PRK activity. Wilson *et al.* [37•] improved the fidelity of this system by fusing PRK with neomycin phosphotransferase, which negated the selection of false positives cells. In another conditional screen, a RuBisCO-dependent *E. coli* selection system was developed utilizing an *E. coli* strain missing the glyceraldehyde-3-phosphate dehydrogenase [35•]. Introduction of PRK and RuBisCO provided a metabolic shunt allowing growth of the engineered strain. This system was used to identify a novel mutation RBC^{F140I} that showed ~3-fold improved carboxylation with only slight reduction in substrate specificity [38••]. Importantly, expression of the RuBisCO variant in *Synechocystis* sp. PCC 6803 improved photosynthesis rate by ~55% using ~25% less amount of RuBisCO [38••]. Finally, an *E. coli* strain missing the phosphoglycerate mutase was used to establish a fully functional CBB cycle that provided all sugar derived metabolites from CO_2 [36••]. This platform could also be used in future studies to engineer RuBisCO for improved carboxylation.

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