Biosensor-informed engineering of *Cupriavidus necator* H16 for autotrophic D-mannitol production

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**ABSTRACT**

*Cupriavidus necator* H16 is one of the most researched carbon dioxide (CO2)-fixing bacteria. It can store carbon in form of the polymer polyhydroxybutyrate(145,245),(934,276) and generate energy by aerobic oxidation under anaerobic conditions, making *C. necator* an ideal chassis for the biological production of value-added compounds from waste gases. Despite its immense potential, however, the experimental evidence of *C. necator* utilisation for autotrophic biosynthesis of chemicals is limited. Here, we genetically engineered *C. necator* for the high-level de novo biosynthesis of the industrially relevant sugar alcohol mannitol directly from Calvin-Benson-Bassham (CBB) cycle intermediates. To identify optimal mannitol production conditions in *C. necator*, a mannitol-responsive biosensor was applied for screening of mono- and bifunctional mannitol 1-phosphate dehydrogenases (MtlDs) and mannitol 1-phosphate phosphatases (M1Ps). We found that MtlD/M1P from brown alga *Ectocarpus siliculosus* performed overall the best under heterotrophic growth conditions and was selected to be chromosomally integrated. Consequently, autotrophic fermentation of recombinant *C. necator* yielded up to 3.9 g/L mannitol, representing a substantial improvement over mannitol biosynthesis using recombinant cyanobacteria. Importantly, we demonstrate that at the onset of stationary growth phase nearly 100% of carbon can be directed from the CBB cycle into mannitol through the glyceraldehyde 3-phosphate and fructose 6-phosphate intermediates. This study highlights for the first time the potential of *C. necator* to generate sugar alcohols from CO2 utilising precursors derived from the CBB cycle.

1. Introduction

The production of chemicals and fuels from renewable biological resources or waste forms a rapidly growing segment of the bioeconomy. In the past decade, considerable progress has been made towards developing microbial cell factories for the conversion of waste gases, such as carbon dioxide (CO2), into value-added compounds (Yunus et al., 2018; Grenz et al., 2019; Panich et al., 2021; Jiang et al., 2021). CO2 is produced during a wide range of natural and industrial processes and acts as a key greenhouse gas responsible for global climate change. One of the UN sustainable development goals is a transition towards net-zero carbon emissions by 2050 (Pavez et al., 2018), for which the utilisation of CO2 as an abundant and low-cost feedstock for the synthesis of chemicals and fuels is hugely beneficial. However, considering the enormous amount of basic and applied research that has been carried out to produce value-added chemicals and fuels in the last few decades, there is only a limited number of examples where thermodynamically stable and highly inert CO2 is utilised as a feedstock in industrially sustainable processes. The large scale transformation of CO2 into valuable products through heterogeneous catalysis requires a significant input of energy and is primarily based on the use of high-energy hydrogen and methane, yielding only a few compounds that are industrially sustainable to produce at large scale, including methane, higher hydrocarbons (Fischer-Tropsch synthesis), methanol, formic acid, and dimethyl ether (Kondratenko et al., 2013; Ye et al., 2019; Ra et al., 2020; Kamkeng et al., 2021). Photo- and electrocatalysis show potential for use in the conversion of CO2 into chemicals and fuels, building on recent technical and catalytic advances that enable improved energy efficiency and productivity (Bushuyev et al., 2018; Jesić et al., 2021), whereas biocatalysis or microbial cell factory-based technologies require further development to become economically viable.

Chemolithoautotrophic *Cupriavidus necator* (formerly known as...
R. eutropha) is capable of fixing CO₂ through a reductive pentose phosphate cycle, i.e. CBB cycle. In the absence of organic substrates, it can utilise CO₂ and H₂ as sole carbon and energy sources. Due to its ability to store large amounts of reduced carbon in the form of poly-hydroxybutyrate (PHB) (Schlegel et al., 1961a; Steinbüchel and Schlegel, 1991), C. necator is considered a promising host organism for the sustainable production of value-added compounds from CO₂. In the last decade, with an extensive genetic toolkit available, allowing genome editing and the controlled expression of heterologous genes, C. necator has been engineered for the autotrophic production of methyl ketones (Müller et al., 2013), alka(e)nes (Crepin et al., 2016), isopropanol (Marc et al., 2017), α-humulene (Kriegl et al., 2018), acetoin (Windhorst and Gescher, 2019), trehalose (Löwe et al., 2021), lipochitooligosaccharides (Nangle et al., 2020), 2,3-butanediol (Bommareddy et al., 2020) and 1,3-butanediol (Gascogne et al., 2021). A majority of these compounds are biosynthesised utilising precursors such as pyruvate and acetyl-CoA derived from the Entner–Doudoroff (ED) pathway.

Under autotrophic growth conditions, hexoses or their derivatives can be directly synthesised from CO₂ and water via the Calvin-Benson-Bassham (CBB) pathway with the energy (ATP) and reducing power (NADH), required for carbon fixation, generated by membrane-bound and soluble hydrogenases (Nybo et al., 2015). D-Mannitol (hereafter denoted mannitol) is an acyclic hexose alcohol that is naturally present in many plant species and can be synthesised by a wide range of microorganisms including bacteria, yeasts, fungi, lichens, and algae (Wiselink et al., 2002). For example, the brown alga Laminaria digitate (also commonly known as oarweed) accumulates mannitol as carbon and energy source up to 20% of its dry weight (Schiener et al., 2015). Mannitol is widely utilised in the food, pharmaceutical, chemical, and medical industries. As a food additive with a glycemic index of zero, it has no significant effects on blood sugar levels and is therefore used as a low-caloric sweetener suitable for diabetics (Song and Vieille, 2009). Moreover, due to its low hygroscopicity, chemical stability and sweet cool taste, which may mask the unpleasant taste of drugs, mannitol is often used in the manufacture of chewing gum, tablets and granulated powders (Patra et al., 2009).

Mannitol can be obtained by extraction, chemical synthesis, and biosynthesis. Extraction of mannitol from plants using supercritical CO₂ or pressurised hot water has been successfully demonstrated (Ghoreishi and Sharifi, 2001; Ghoreishi and Shahrestani, 2009). Its application, however, is limited by the availability of raw material and seasonal variation in mannitol content (Schiener et al., 2015). Currently, mannitol is produced industrially by catalytic hydrogenation of fructose/glucose mixtures with sorbitol as a byproduct (Wislak and Simon, 1979). Although significant progress has been made towards developing catalysts with higher selectivity to mannitol (Zelin et al., 2019), separation from its isomer sorbitol and chromatographic removal of the metal catalyst remain challenging. The biological production of mannitol is mainly achieved by fermentation of lactic acid bacteria Lactobacillus intermedius and yeast Candida magnoliae using fructose and glucose as carbon sources resulting in high mannitol titres (>100 g/L), productivities (>4 g/L/h) and yields (>0.5 (C-mol mannitol)/(C-mol hexoses)) (Racine and Saha, 2007; Saveridge et al., 2013). Besides, similarly high titres, productivities and yields have been achieved through whole-cell biotransformation of glucose and fructose using recombinant Escherichia coli and Corynebacterium glutamicum, respectively (Kaup et al., 2005; Baumbach and Brünger-Meyer, 2007).

In this study, we used C. necator H16 as a microbial chassis and as a whole cell biosensor to evaluate monofunctional mannitol 1-phosphate dehydogenases (MtlDs) and mannitol 1-phosphate phosphatases (M1Ps), as well as bifunctional MtlD/M1Ps originating from several kingdoms of life for mannitol biosynthesis. A mannitol biosensor was applied not only to screen the combination of genes that enables the highest production of mannitol, but also to identify optimal conditions for gene expression induction. For stable production of mannitol, C. necator was engineered by integration of mannitol biosynthesis genes into two different genomic loci, including PHB biosynthesis inactivation. The autotrophic production of mannitol using CO₂ as sole carbon source was demonstrated in batch fermentations yielding mannitol titres, productivities and yields that are substantially higher than previously reported for other CO₂-fixing bacteria. This works highlights the potential of C. necator to be used as chassis organism for the economically viable conversion of CO₂ into sugar alcohols.

2. Materials and methods

2.1. Base strains and media

E. coli DH5α (New England Biolabs, NEB) was employed for cloning and plasmid propagation. E. coli S17-1 pir was used as conjugation donor. C. necator H16 was used for heterologous expression of mannitol biosynthetic genes. All bacterial strains used in this study are listed in Table 1. Both E. coli and C. necator were routinely grown in lysesogeny broth (LB)(Sambrook and Russell, 2001). Low-salt LB (LSLB)-MOPS was used for conjugative plasmid transfer and gene replacement (Lenz et al., 1994). The initial screening of genes for mannitol biosynthesis in C. necator and the heterotrophic production of mannitol in small volumes were performed in chemically defined minimal medium (MM) (Schlegel et al., 1961b) supplemented with 1 mL/L trace element solution SL7 (Trüper and Pfennig, 1981) and 0.4% (w/v) sodium-glucuronate as carbon source. The mannitol biosensor-assisted investigation of inducer range and the heterotrophic production of mannitol in shake flasks were performed in LB medium. Pre-cultures for bioreactor cultivations were set up in Hanahan’s broth (SOB medium) (Hanahan, 1983). Autotrophic production of mannitol was performed in modified DSMZ 81 medium (Bommareddy et al., 2020). When appropriate, the medium was supplemented with the following antibiotics: tetracycline (12.5 μg/mL for E. coli or 15 μg/mL C. necator), chloramphenicol (25 μg/mL for E. coli or 50 μg/mL C. necator), or gentamycin (10 μg/mL). For solid media preparation, 15 g/L agar was added. All chemicals, including media components, were purchased from Sigma-Aldrich unless indicated otherwise.

2.2. Cloning and transformation

Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen). Bacterial genomic DNA was isolated using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich). Oligonucleotide primers were synthesised by Sigma-Aldrich and are listed in Supplementary Table 1. DNA for cloning was amplified by PCR in 50 μL reactions using Phusion High-Fidelity DNA polymerase (NEB). Screening of bacterial colonies for successful assembly of vectors was performed using DreamTaq Green

<table>
<thead>
<tr>
<th>Strain</th>
<th>Parental strain</th>
<th>Genotype/description</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli DH5α</td>
<td>–</td>
<td>fhuA2 (argF-leu2)-lacZ169 phoA glnV44 80 (lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hisD17</td>
<td>NRB</td>
</tr>
<tr>
<td>S17-1</td>
<td>pir</td>
<td>thi pro hisR indB7 recA RP4-2-Tc: Mu-Km::Tn5 pir Tn5 Sm^</td>
<td>Simon et al. (1983)</td>
</tr>
<tr>
<td>C. necator H16</td>
<td>–</td>
<td>Wild type strain (DSM 428)</td>
<td>DSMZ</td>
</tr>
<tr>
<td>NM0010 H16</td>
<td>–</td>
<td>ΔphaCAB, P_pscE2-araC, P_pscE2-mtlD/m1p</td>
<td>This study</td>
</tr>
<tr>
<td>NM0011 H16</td>
<td>–</td>
<td>ΔcruAB, P_pscE2-araC, P_pscE2-mtlD/m1p</td>
<td>This study</td>
</tr>
<tr>
<td>NM0013 H16</td>
<td>–</td>
<td>ΔcruAB, P_pscE2-mtlD/m1p</td>
<td>This study</td>
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a m1p/D/m1p: N-terminal truncated version of MtlD and M1P2 from E. siliculosus.
PCR Master Mix (2X, Thermo Fisher Scientific) in 25 μL reactions. Gel-purified linearised DNA was extracted using the Zymoclean Gel DNA Recovery Kit. Restriction enzymes, T4 DNA ligase and the NEBuilder HiFi DNA Assembly Master Mix were purchased from NEB. PCR-, digestion-, and ligation reactions were set up following the manufacturer’s instructions. Chemical competent E. coli were prepared and transformed by heat shock as described by Sambrook and Russell (2001). C. necator cells were prepared and transformed by electroporation following a method reported by Ausubel et al. (2003) or by conjugation (Lenz et al., 1994).

2.3. Plasmid construction and gene integration

Plasmids were constructed by HiFi DNA assembly or conventional restriction enzyme-based cloning (Sambrook and Russell, 2001). The P. putida mtlD/m1p was amplified from P. putida KT2440 genomic DNA. EsM1PDH1cat (here termed EsmID) and EsM1Pase2 (here termed EsmIP) were amplified from pESM1PDH1cat and pFESM1Pase2, respectively (Bonin et al., 2015; Groisillier et al., 2014). The remaining mtlD/m1p genes were optimised for E. coli codon usage and synthesised (Thermo Fisher Scientific). Coding sequences can be found in Supplementary Table 3. The plasmids harbouring the mannitol production-detection systems contain: the genes encoding the mannitol- and arabinose-responsive transcriptional regulators MtlR and AraC, respectively, under control of their native promoters (Hoffmann and Altenbuchner, 2015; Schleif, 2000), the rfp reporter gene under control of the mannitol-inducible promoter Pm1p, and the arabinose-controllable promoter ParaBAD (Supplementary Fig. 1). The version of Pm1p used in this study harbour a mutation in the −35 element resulting in a strong reduction in basal promoter activity whilst maintaining a high induction ratio (Hoffman and Altenbuchner, 2015). To enhance mtlD/m1p gene expression through improved mRNA stability, the bacteriophage T7 gene 10 (T7g10) 5’ untranslated region was introduced upstream of the E. coli consensus RBS sequence “tttaaagggagatatcat” (Bi et al., 2013).

Plasmids that were used for genomic integration of the mannitol biosynthesis pathway are derivatives of the suicide plasmid pLO3 (Lenz and Friedrich, 1998). They harbour E. siliculosus mtlD/m1p under control of the heterologous arabinose-inducible system or the native phaC promoter, flanked by homology arms of roughly 1000 bp each, being identical to the upstream and downstream regions of the integration site (Supplementary Fig. 2). Gene integrations were performed following the method described by Lenz and co-workers (Lenz et al., 1994).

All constructs were verified by PCR and Sanger sequencing (Source BioScience, Nottingham). A detailed assembly description for each plasmid is provided in the Supplementary Methods. Key features of all plasmids used and generated in this study are summarised in Supplementary Table 3. The nucleotide sequences of the plasmids pEH030, pEH031, pEH067, pEH140, pEH141, pEH142 and pEH145 have been deposited in the public version of the ACS registry (https://acs-registry.jbei.org) under the accession numbers ACS_000867 to ACS_000873, respectively.

2.4. Cultivation in small-volume cultures

For the initial screening of genes for mannitol biosynthesis in C. necator and the heterothrophic production of mannitol in small volumes, individual colonies of freshly transformed C. necator were used to inoculate 5 mL of MM containing chloramphenicol in 50-ml conical centrifuge tubes. Following incubation overnight with orbital shaking at 200 rpm and 30 °C, cells were diluted to an OD\textsubscript{600} of 0.25 in fresh 10 mL of MM containing the respective antibiotic. After incubation for 1 h, each culture was split into two equal parts. L-arabinose was added to one culture to a final concentration of 0.02% (wt/vol), whereas the other one remained non-induced. As before, cells were incubated at 30 °C with orbital shaking at 200 rpm. Samples of 0.5 mL were taken 6, 24, and 48 h after supplementation with arabinose and used to quantify culture OD\textsubscript{600}, RFP fluorescence and mannitol concentration using high performance liquid chromatography (HPLC).

2.5. Investigation of inducer range

For the mannitol biosensor-assisted investigation of inducer range, individual colonies of freshly transformed C. necator were used to inoculate 5 mL of LB medium containing chloramphenicol in 50-ml conical centrifuge tubes. Following incubation overnight with orbital shaking at 200 rpm and 30 °C, cells were diluted to an OD\textsubscript{600} of 0.05 in fresh 5 mL of LB containing the respective antibiotic. After incubation for 2 h, 142.5 μL of culture were transferred to a well of a 96-well clear-bottom plate (Greiner Bio-One International). To each well, 7.5 μL of arabinose stock solution were added to obtain the desired inducer concentration. Cells were incubated in an Infinite M1000 PRO micro plate reader (Tecan) with orbital shaking at 582 rpm and an amplitude of 1 mm and the temperature set at 30 °C. RFP fluorescence and culture OD\textsubscript{600} were measured from cells in late exponential growth phase after 8 h.

2.6. Cultivation in large-volume cultures and extraction of intracellular mannitol

For the heterotrophic production of mannitol in large volumes, pre-cultures were set up as for the mannitol biosensor-assisted investigation of inducer range. The overnight culture was diluted 1:50 into fresh LB medium containing chloramphenicol. Cultures were grown in 50-ml volumes in 250-ml baffled shake flasks with orbital shaking at 200 rpm and 30 °C. At an OD\textsubscript{600} of 0.2-0.4, 100 μL of arabinose stock solution were added to obtain the desired inducer concentration. Samples were taken 12 h after supplementation with arabinose and used to quantify culture OD\textsubscript{600} and mannitol concentration using HPLC. To determine the intracellular mannitol concentration, the culture volume corresponding to an OD\textsubscript{600} of 50 was centrifuged for 10 min at 16,000g. The cell pellet was washed in 1 mL of phosphate buffered saline, transferred to a microcentrifuge tube and centrifuged as before. Subsequently, the supernatant was completely removed, the pellet was weighed using fine balance and frozen overnight at −80 °C. Extraction and calculation of intracellular mannitol concentration was performed as described previously (Hanko et al., 2018).

2.7. Cultivation in bioreactors

Autotrophic batch fermentation of C. necator was performed as reported previously by Gascoyne and co-workers (Gascoyne et al., 2021) with slight modifications. Briefly, to prepare the fermenter culture, individual colonies of freshly streaked C. necator were used to inoculate 5 mL of LB medium containing gentamycin in 50-ml conical centrifuge tubes. To set up the fermenter inoculum, 1 mL of overnight culture was transferred into 120 mL of SOB medium containing gentamycin. Following incubation overnight with orbital shaking at 200 rpm and 30 °C, cells were harvested by centrifugation at 10,000g for 10 min, washed once in 40 mL of modified DSMZ 81 medium (Bommareddy et al., 2020), centrifuged as before and resuspended in 50 mL of modified DSMZ 81 medium. Subsequently, the resuspended cells were used to inoculate the bioreactor with a total working volume of 750 mL. Expression of E. siliculosus mtlD/m1p in strains NM0010 and NM0011 was induced by addition of arabinose to a final concentration of 5 mM at dry cell weights (DCWs) greater than 1 g/L. To limit nitrogen availability the base was switched from 1 M NH\textsubscript{4}OH to 1 M KOH at DCWs of 2 g/L.

2.8. Fluorescence measurements

To quantify RFP fluorescence, unless directly grown in a microtitre plate, 100 μL of cells were transferred to a 96-well clear bottom plate
and fluorescence and OD_{600} were quantified using an Infinite M1000 PRO micro plate reader. Fluorescence excitation and emission wavelengths were set to 585 nm and 620 nm, respectively. For fluorescence measurements in minimal medium the gain factor was set manually to 100%, whereas for rich medium it was set to 80% unless indicated otherwise. Culture optical density was measured at 600 nm to normalise RFP fluorescence by optical density. To account for media auto-fluorescence and -optical density, fluorescence and OD_{600} values were corrected by subtracting the fluorescence and OD_{600} of the cell-free culture medium prior to normalisation.

### 2.9. Analytical methods

Samples for HPLC analysis were prepared by combining cell-free supernatants with an equal volume of mobile phase spiked with 50 mM valerate as internal standard. The mobile phase was composed of 5 mM H_{2}SO_{4}. Subsequently, the mixture was passed through a cellulose acetate syringe filter with a pore size of 0.22 μm. Samples were analysed using a Thermo Scientific UtiMate 3000 HPLC system equipped with a Phenomenex Rezex ROA-organic acid 8% column, a diode array detector DAD-3000 with the wavelengths set at 210 and 280 nm and a refractive index detector RefractoMax 521 (Thermo Fisher Scientific). The flow rate of the mobile phase was set to an isocratic 0.5 mL/min with a column temperature of 35 °C. Samples were run for 30 min and the injection volume was 20 μL. Data analysis was performed using Chromelone 7 (Thermo Fisher Scientific). Mannitol concentrations were quantified using calibration curves generated from running standards of known concentrations, which were prepared the same as the samples.

DCW was quantified by pelleting 1 mL of cell culture in a pre-dried and pre-weighed 1.5 mL Eppendorf tube. The supernatant was removed, and the cell pellet was dried for 48 h at 100 °C and weighed using a fine balance. DCW was calculated as grams per litre.

### 2.10. Mathematical modelling

To obtain biosensor dose-response parameters, normalised fluorescence values were plotted as a function of mannitol concentration using software GraphPad Prism 7. A non-linear least-squares fit was performed using Hill function:

\[
RFP(I) = \frac{b_{\text{max}} \times I^h}{K_m + I} + b_{\text{min}}
\]

Parameters correspond to the maximum level of biosensor output (b_{max}), the concentration of mannitol (I), the Hill coefficient (h), the mannitol concentration mediating half-maximal biosensor output (K_m), and the basal level of fluorescence output (b_{min}).

### 3. Results

#### 3.1. Screening of genes for mannitol biosynthesis in C. necator

The biosynthesis of mannitol from fructose 6-phosphate (F-6-P) involves two catalytic steps (Fig. 1A). First, F-6-P is reduced to mannitol 1-phosphate (Mtl-1-P) by the action of a Mtl-1-P dehydrogenase (MtlD, also commonly referred to as MIPDH). Subsequently, Mtl-1-P is dephosphorylated by Mtl-1-P phosphatase (MIP, also referred to as MIPase), forming mannitol. This mannitol biosynthetic pathway from F-6-P, involving two independent enzymes, has been characterised in homofermentative lactic acid bacteria (Neves et al., 2000), algae (Iwamoto et al., 2003), and Apicomplexa (Schmattz et al., 1989). Recently, an MIPD/MIP fusion protein from Acinetobacter baylyi ADP1 has been shown to catalyse both enzymatic reactions, F-6-P reduction and Mtl-1-P dephosphorylation (Sand et al., 2015). Bioinformatic analysis of the bifunctional enzyme revealed a haloacid dehalogenase-like phosphatase domain at the N-terminus being unique to previously reported MtlDs (Sand et al., 2015). As enzymes with the same catalytic function from different organisms often exhibit distinct catalytic efficiencies, we sought to test a number of combinations of monofunctional MtlDs/M1Ps, as well as bifunctional fusion MtlD/M1Ps for their ability to produce mannitol in C. necator (Supplementary Table 4).

The first pair of monofunctional enzymes that was tested for mannitol production originates from the model brown alga Ectocarpus siliculosus. Previously, its N-terminal domain of 142 amino acids was shown to be non-essential for E. siliculosus MtlD enzymatic function (Bonin et al., 2015; Rousvoal et al., 2011). Therefore, a truncated version of E. siliculosus mtlD, reduced to solely encode the catalytic domain of the enzyme (Bonin et al., 2015; Rousvoal et al., 2011), was co-expressed with E. siliculosus m1p2 (Groisillier et al., 2014). The second pair of monofunctional enzymes was sourced from the protozoan...
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chicken parasite *Eimeria tenella*. *E. tenella* M1P was previously employed in combination with the *Escherichia coli* MtdD for the production of mannitol in *Synechococcus sp*. PCC 7002 (Jacobson and Frigaard, 2014) and *E. coli* itself (Reshamwala et al., 2014). Here, mannitol biosynthesis was investigated upon expression of *E. tenella* m1p in combination with *E. tenella* mtdD. Due to a sequence of poly-serines, most likely resulting in poor translational efficiency, and protein sequence homology to the non-catalytic N-terminal domain of *E. siliculosus* MtdD (Rousvoal et al., 2011), the *E. tenella* MtdD was truncated by 43 residues ranging from A438 to L479 and by 168 residues at the N-terminus, respectively. Deletion of amino acids was performed in such way that the *E. tenella* MtdD protein sequence aligns with *E. siliculosus* MtdD and other species homologues without gaps in the sequence region where amino acids were removed. The bifunctional enzymes that were investigated for mannitol biosynthesis were obtained from the following organisms: the soil bacteria *Pseudomonas putida*, *Acinetobacter baylyi*, and *Closstridium pasteurianum*, as well as the green alga *Micromonas pusilla*. The bifunctional enzyme of the latter has been demonstrated to facilitate mannitol production in both *E. coli* and *Synechococcus sp*. PCC 7002 (Vladise et al., 2018). As for *E. tenella* MtdD, the *M. pusilla* MtdD/M1P was truncated by 73 residues at the N-terminus to align with the catalytic domain of *E. siliculosus* MtdD.

To facilitate mono- and bifunctional MtdD/M1P screening under different expression conditions, corresponding genes were cloned into vector pEH031 (Supplementary Table 3, Supplementary Fig. 1) that contains a mannitol biosensor, enabling the *in vivo* monitoring of mannitol formation by red fluorescent protein (RFP) fluorescence output. Implementation of a mannitol biosensor offers the advantage that individual parameters affecting product biosynthesis, including enzyme turnover number or gene expression level, can be rapidly evaluated. Expression of the mannitol biosynthesis pathway is controlled by the L-arabinose-inducible system (Fig. 1B). Supplementation of the growth medium with arabinose initiates expression of mtdD/m1p, the translational product/s of which catalyse/s the conversion of F-6-P to mannitol. Subsequently, the mannitol-responsive transcriptional regulator MtlR activates expression of the rfp reporter gene from its corresponding promoter pMttD (Hoffmann and Altenbuchner, 2015). To gain an initial overview of which enzymes catalyse the biosynthesis of mannitol, *C. necator* cells carrying the mannitol production-sensor vectors were grown in the absence and presence of arabinose and RFP fluorescence was monitored. All constructs harbouring mtdD/m1p genes showed a statistically significant induction of reporter gene expression 24 h after supplementation with arabinose (Fig. 1C). The highest level of fluorescence was achieved by *E. siliculosus* MtdD/M1P, followed by *P. putida* MtdD/M1P, exceeding fluorescence levels of the other enzyme candidates tested by more than 5-fold. Except for *E. siliculosus* and *A. baylyi* MtdD/M1P, resulting in considerable levels of background fluorescence output, reporter gene expression in the absence of arabinose was comparable to the negative control plasmid lacking mtdD/m1p. The functionality of the mannitol-sensor module was confirmed by extracellular addition of D-mannitol to cells carrying the negative control plasmid pEH031. Whereas supplementation of the growth medium with 0.2% (wt/vol) mannitol led to a 97-fold increase in RFP fluorescence (Supplementary Fig. 3), addition of arabinose to cells carrying pEH031 did not induce reporter gene expression (Fig. 1C, Supplementary Fig. 3). This suggests that an increase in fluorescence output in response to arabinose is caused by biosynthesis of mannitol rather than expression of the mannitol sensor by arabinose. Moreover, to determine its detection range, cultures of *C. necator* solely carrying the mannitol biosensor, pEH003, were subjected to a range of mannitol concentrations and RFP fluorescence and optical density at 600 nm (OD600) were quantified for cells in exponential growth phase. The dose-response was obtained by plotting the normalised fluorescence values as a function of mannitol concentration and data points were fit using a Hill function (see Methods). When extracellularly added to cells harbouring the biosensor, the mannitol concentration that mediated half-maximal biosensor output, Kₘ, was 3.7 mM (Supplementary Fig. 4). The concentrations of mannitol falling within 10% and 90% of the maximum level of biosensor output, b_max, ranged from 1.2 mM to 9.7 mM. It should be noted, however, that the amount of mannitol with which the medium was supplemented may differ from the intracellular mannitol concentration mediating the biosensor output.

3.2. Heterotrophic production of mannitol in small volumes

To confirm the observations of the initial enzyme screen, mannitol titres were quantified in cultures of *C. necator* carrying the various mannitol production-sensor vectors. Cells were grown in 5-mL cultures of minimal medium with 0.4% (wt/vol) gluconate as sole carbon source and expression of mtdD/m1p was induced by addition of L-arabinose to a final concentration of 0.02% (wt/vol). Mannitol could be detected in the culture supernatants of all strains tested. However, final mannitol titres varied greatly from 7 mg/L (*M. pusilla* MtdD/M1P) to 429 mg/L (*E. siliculosus* MtdD/M1P) with the latter strain (Fig. 2) resulting in a molar yield of 0.13 (mol mannitol)/(mol gluconate) at 24 h. Whereas mannitol titres remained below the detection limit until the 24 h after addition of arabinose for cells expressing mtdD/m1p from *E. tenella*, *C. pasteurianum*, and *M. pusilla*, mannitol could be detected after 6 h in the cultures of the three best performing strains producing *E. siliculosus*, *P. putida*, and *A. baylyi* MtdD/M1P. In addition, mannitol was produced by these three strains even in the absence of arabinose with cells expressing *E. siliculosus* mtdD/m1p forming 21.6 mg/L mannitol at the 24 h time point (Supplementary Table 5).

3.3. Mannitol biosensor-assisted investigation of inducer range

Using inducible promoters for controlling expression of biosynthetic pathways offers the advantage that the level of gene expression can be fine-tuned as it is a function of inducer concentration. Balancing gene expression is often crucial to avoid negative effects from accumulated intermediate products and to ensure optimal metabolic flux (Lee et al., 2013). Here, expression of the heterologous mtdD/m1p gene/s is mediated by the arabinose-inducible system, which was previously shown to be highly operable in the range between 0.016 and 2.5 mM in *C. necator* (Alagesan et al., 2018a). To determine the effect of varying inducer concentrations on both mannitol biosensor output and cell viability, strains of *C. necator* carrying the various mannitol production-detection systems were subjected to a wide range of arabinose concentrations and RFP fluorescence and OD₆₀₀ were quantified for cells in late exponential growth phase. In contrast to the initial screen of enzyme candidates (Fig. 1C), which was performed in minimal medium with gluconate as carbon source, the investigation of arabinose-inducer range was conducted in rich medium. It should be noted that the inducer concentration, at which the induction rate of the arabinose-inducible system is 50%, is similar for *C. necator* cells grown either in minimal or rich medium as reported previously (Alagesan et al., 2018a; Johnson et al., 2018). Moreover, considering that in response to a higher availability of nutrients cells in rich medium generally grow faster and the expression of housekeeping genes, including translation apparatus genes, is elevated (Tao et al., 1999), the use of rich medium enabled to establish whether the biosynthesis of mannitol can be improved by enriching growth medium and validate the best mannitol production systems.

For cells grown in complex medium, two different biosensor output patterns could be observed (Fig. 3). In the absence of arabinose, cells expressing mtdD/m1p from *E. tenella*, *P. putida*, and *M. pusilla* exhibited fluorescence outputs at the level of the negative control vector (Supplementary Table 6). An increase in arabinose concentrations up to 10 mM generally led to an increase in normalised fluorescence, with the exception of cells producing *E. tenella* MtdD/M1P demonstrating maximum normalised fluorescence levels at 1.25 mM arabinose. In contrast, the biosensor output resulting from expression of *E. siliculosus*, *A. baylyi*, and *C. pasteurianum* mtdD/m1p was constant within the range of background fluorescence output, Kₘ, was 3.7 mM (Supplementary Fig. 4). The concentrations of mannitol falling within 10% and 90% of the maximum level of biosensor output, b_max, ranged from 1.2 mM to 9.7 mM. It should be noted, however, that the amount of mannitol with which the medium was supplemented may differ from the intracellular mannitol concentration mediating the biosensor output.
of error for all arabinose concentrations tested. Maximum fluorescence levels normalised by culture OD_{600} for the individual MtlD/M1Ps (and their corresponding arabinose concentrations) were as follows: E. tenella, 3678 ± 277 (1.25 mM); E. siliculosus, 9231 ± 1890 (10 mM); P. putida, 7087 ± 1144 (10 mM); A. baylyi, 6509 ± 457 (0.156 mM); C. pasteurianum, 7692 ± 1205 (0.156 mM); M. pusilla, 9526 ± 1293 (5 mM).

A similar effect on cell growth could be observed for all mannitol biosynthetic genes with an increase in arabinose concentration resulting in reduced cell density (Fig. 3). Supplementation of wild type C. necator cells with 10 mM arabinose did not affect cell growth (data not shown). Expressing the different candidate genes using a range of inducer concentrations, however, reduced cell viability from 19% (P. putida mtlD/m1P) to up to 84% (M. pusilla mtlD/m1P, at 5 mM arabinose).

3.4. Heterotrophic production of mannitol in large volumes

Based on the results of the initial enzyme screen (Fig. 2), the three best performing strains were selected to be analysed for mannitol biosynthesis in heterotrophic shake flask cultures. Informed by the biosensor-assisted evaluation of inducer range, mannitol production was quantified in response to three different inducer concentrations. They
were chosen for each strain individually to reflect what was presumed to be the optimum level of induction, exhibiting a high fluorescence output whilst maintaining a high cell viability (e.g. 0.625 mM, 0.625 mM, and 0.156 mM arabinose for *P. putida*, *E. siliculosus* and *A. baylyi mtlD/m1p*, respectively), as well as one inducer concentration below and one above this level. *C. necator* carrying the mannotol production-detection vectors was grown in 50 mL of LB. Arabinose was added to exponentially growing cells (OD_{600} of 0.2–0.4) and samples were taken 12 h after inducer addition. To investigate the cells’ ability to export the final product, mannotol was quantified in both culture supernatant and cell pellet.

In all three strains tested, supplementation of the growth medium with the highest arabinose concentration resulted in the highest mannotol titre (Fig. 4A). With the exception of *P. putida mtlD/m1p* expressed at 0.156 mM arabinose, showing a 52/48 split between the extra- and intracellular fraction, the majority of mannotol of at least 80% was found in the culture supernatants (Fig. 4B). Mannotol titres at the highest arabinose concentrations tested were 54 mg/L, 245 mg/L, and 277 mg/L in culture supernatants of cells expressing mtlD/m1p from *P. putida*, *E. siliculosus*, and *A. baylyi*, respectively. Similar to what has been observed for cells grown in small volumes of minimal medium (Supplementary Table 5), mannotol could be detected in cultures of cells carrying *E. siliculosus* and *A. baylyi mtlD/m1p* in the absence of arabinose. These results are consistent with the outcome of the biosensor-assisted investigation of inducer range where *C. necator* expressing *E. siliculosus* and *A. baylyi mtlD/m1p* demonstrated a considerable fluorescence reporter output even under non-inducing conditions (Supplementary Table 6). As the leakiness from the arabinose-inducible promoter may be considered the same between all gene candidates tested, biosynthesis of mannotol under non-inducing conditions suggests a higher translational or catalytic efficiency of *E. siliculosus* and *A. baylyi MtlD/M1P*. Because of its overall performance in both minimal and complex media, mtlD/m1p from *E. siliculosus* was selected to be analysed for autotrophic production of mannotol in batch fermentations.

### 3.5. Autotrophic production of mannotol

To investigate whether F-6-P, generated via the Calvin-Benson-Bassham (CBB) cycle during autotrophic growth, can be redirected toward mannotol biosynthesis, *E. siliculosus mtlD/m1p* was integrated into the chromosome of *C. necator*. In total, three strains were generated (Table 1, Supplementary Fig. 2). In strain NM0010, the phaCAB operon, encoding the proteins for the biosynthesis of PHB, was replaced by *E. siliculosus mtlD/m1p* under control of the heterologous arabinose-inducible system. To investigate the impact of constitutive expression of the mannotol biosynthetic genes on product formation, *E. siliculosus mtlD/m1p* were cloned downstream of the native phaC promoter, thereby replacing the phaCAB operon, yielding strain NM0013. A second integration site was selected to determine if the presence of *C. necator*’s natural carbon sink pathway to PHB had any adverse effects on mannotol production. Consequently, the *tcuAB* operon, encoding proteins potentially involved in tricarboxylate catabolism, was replaced by *E. siliculosus mtlD/m1p* under control of the arabinose-inducible system, yielding strain NM0011. In contrast to NM0010 and NM0013, in strain NM0011 the phaCAB operon is intact.

Bioreactors were inoculated at an optical density of 1 with cells resuspended in mineral medium for chemolithotrophic growth. Both H₂ and CO₂ were fed at a rate of 35.1 L/h and 1.35 L/h, respectively. O₂ was fed through air at a variable rate controlled by the off-gas O₂ concentration, which was set at 4% (v/v). To induce expression of *E. siliculosus mtlD/m1p* in strains NM0010 and NM0011, arabinose was added to cells in exponential growth phase to a final concentration of 5 mM. After 167 h of autotrophic fermentation, the final concentrations of dry cell weight (DCW) were 5.5 g/L, 14.2 g/L, and 4.9 g/L for strains NM0010, NM0011, and NM0013, respectively (Fig. 5A). Strains NM0010 and NM0013 reached a maximum concentration of DCW after 71 h, whereas in the PHB-producing strain, NM0011, biomass continued to increase until the 143 h time point. Mannotol was produced in all three strains as indicated by the absolute mannotol titres and specific productivity on a biomass basis (Fig. 5B and C). Strain NM0013 (AphaCAB P_{phaCAB-mtlD/m1p}) started producing mannotol immediately after inoculation and exhibited a constant level of productivity ranging from 0.036 [(mmol mannotol)/(g DCW⋅h)] to 0.054 [(mmol mannotol)/(g DCW⋅h)] between 17 h and 71 h. The strains NM0010 (AphaCAB P_{araBAD-mtlD/m1p}) and NM0011 (AphaCAB P_{araBAD-mtlD/m1p}) reached a maximum specific productivity of 0.069 [(mmol mannotol)/(g DCW⋅h)] and 0.06 [(mmol mannotol)/(g DCW⋅h)], respectively, roughly 30 h after arabinose had been added, followed by a slow decrease in productivity until the end of cultivation. Final mannotol titres were 3.9 g/L, 3.3 g/L, and 2.6 g/L for strains NM0010, NM0011, and NM0013, respectively. This represented final yields of 0.71 [(g mannotol)/(g DCW)], 0.23 [(g mannotol)/(g DCW)], and 0.53 [(g mannotol)/(g DCW)], respectively, after 167 h of gas fermentation. From 53.5 h to 71 h, strains NM0010, NM0011, and NM0013 achieved molar carbon yields of 0.48 [(C-mol mannotol)/(C-mol CO₂)], 0.16 [(C-mol mannotol)/(C-mol CO₂)] and 0.26 [(C-mol mannotol)/(C-mol CO₂)] (Fig. 5D), respectively, and increased further.

![Fig. 4.](image-url) Mannitol biosynthesis in heterotrophic shake flask cultures of *C. necator* carrying plasmids pH030, pH067 and pH140 with mtlD/m1p from *P. putida*, *E. siliculosus*, and *A. baylyi*, respectively, under control of the arabinose-inducible system. **A** Specific production, **B** Mannitol split between culture supernatant and cell pellet (in % of total). Cells were grown in 50 mL of LB supplemented with arabinose at the concentrations indicated. Samples were taken 12 h after addition of arabinose and mannotol was quantified. No mannotol was detected in cells carrying *P. putida mtlD/m1p* in the absence of arabinose. Results are the average of three biological replicates with error bars representing the standard deviation from the mean.
throughout the cultivation as mannitol titres continued to rise. In the time frame 71 h–95 h, the specific CO$_2$ uptake rate dropped significantly during fermentation of strain NM0010 (Supplementary Fig. 5). This was coupled with a decrease in biomass and an increase in mannitol titre (Fig. 5 A and B), consequently resulting in a molar carbon yield of 2.74 [(C-mol mannitol)/(C-mol CO$_2$)]. It should be noted that no extracellular by-products were observed for any of the strains throughout their cultivation.

4. Discussion

The utilisation of CO$_2$ as a feedstock for the biosynthesis of chemicals and fuels is critically important seeking to reduce greenhouse gas emissions and re-cycle combustible waste. However, considering the enormous amount of basic and applied research that has been carried out on the bioproduction and biotechnology of chemical compounds, there is a limited number of examples where CO$_2$ is utilised as a carbon source. Nonetheless, alongside cyanobacteria, chemolithoautotrophic bacterium C. necator H16 has attracted significant attention for its ability to accumulate large quantities of PHB under excess carbon and utilise CO$_2$ as sole carbon source.

Mannitol is industrially either extracted from seaweed or produced chemically by hydrogenation of fructose. It is ranked as one of the top twenty bio-based chemical opportunities in the UK (E4tech (UK) Ltd for LBNet, 2017). In this study, C. necator H16 transformed with a set of plasmids was utilised as a microbial chassis and whole cell biosensor to screen several mannitol biosynthesis genes. Of the investigated genes E. siliculosus, A. baylyi, and P. putida mtlD/m1p variants were identified as most prominent for mannitol production in C. necator H16. Subsequently, we engineered C. necator H16 by integrating mtlD and m1p2 from E. siliculosus for autotrophic production of mannitol from CO$_2$. Three engineered strains achieved final titres from 2.6 to 3.9 g/L.
mannitol. The highest producing strain showed an average productivity of 94.2 mg/L/h and yield of 0.48 ((C-mol mannitol)/(C-mol CO2)) from 53.5 h to the 71 h time point of fermentation. These titles, productivity and yield are substantially higher than those reported for mannitol production from CO2 in recombinant cyanobacteria (Table 2) and they are in the same range with the high productivities of C. necator H16 reported for chemicals such as acetoin (Windhorst and Gescher, 2019), 1,3-butanol (Gascocyte et al., 2021), 2,3-butanol (Bommereddy et al., 2020) and isopropanol (Marc et al., 2017). The mannitol yield was improved in the ΔphaCAB strains (Fig. 5D). Inactivation of PHB biosynthesis resulted in a relatively lower biomass production whilst increasing CO2 conversion into mannitol by 2-3-fold. From the 71 h time point the molar carbon yield of strains NM0010 and NM0013 increased further and significantly above 0.48 ((C-mol mannitol)/(C-mol CO2)) throughout the fermentation as mannitol titres continued to rise. Similarly, as reported for acetoin (Windhorst and Gescher, 2019), the abolishment of PHB synthesis enabled to engineer a strain that was able to produce mannitol with a maximum molar carbon yield close to 1.0 ((C-mol mannitol)/(C-mol CO2)). It should be noted that a transient increase of estimated molar carbon efficiency above theoretical maximum was observed between 71 h and 95 h for strain NM0010 most likely due to the decrease of cell optical density and potential utilisation of the intracellular carbon accumulated in the early phase of fermentation.

The use of the CBB cycle for CO2 fixation allows theoretically up to 100% carbon efficiency during mannitol biosynthesis as the pool of glyceraldehyde 3-phosphate (G-3-P), available for fructose 6-phosphate formation, is continuously replenished. This is supported by reverse activity (gluconeogenesis) of the upper part of the Embden-Meyerhof-Parnas (EMP) pathway as a consequence of the absence of phosphofructokinase in C. necator H16 (Alagesan et al., 2018b). Consistent with this, other products derived from pyruvate, including acetoin (Windhorst and Gescher, 2019), 1,3-butanol (Gascocyte et al., 2021), or 2,3-butanediol (Bommereddy et al., 2020), also showed relatively high titres in engineered C. necator H16. Nonetheless, a relatively low turn-over number of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) limits the rate of productivity. An improved titre of autotrophic mannitol production was achieved by the introduction of the most active combination of mannitol 1-phosphate dehydrogenases and mannitol 1-phosphate phosphatases, which were identified on the basis of a rigorous comparison of enzymes from bacteria and protista. They exhibited significant differences in either catalytic activities, expression levels or both, resulting in a large variation of mannitol biosynthesis in C. necator H16 (Figs. 1C and 2).

To establish whether the achieved maximum productivity of 94.2 mg/L/h is practical, we calculated that 710 g of mannitol can be produced per 1 kg of bacterial DCW in a 0.2 m3 bioreactor within one week. Compared with mannitol production from seaweed, with only 300 g extracted from 1 kg of seaweed DCW, cultivation of which would require 30 m2 of near-shore sea area based on previous estimations (Wei et al., 2013; Milledge et al., 2019), the mannitol productivity of engineered C. necator H16 is very promising. Although the hydrogenotrophic energy generation process used by this bacterium may not be as efficient as in acetogens (Nybo et al., 2015), it can be approximately 5 times higher than the phototrophic energy generation process. It has been estimated that the C. necator H16-based electrolysytic conversion gives a solar-to-product efficiency of approximately 7.6%, whereas solar-to-product conversion efficiencies of photosynthetic species is approximately 1.5% (Claassen et al., 2016).

Here we also demonstrated that the application of a fluorescence-based biosensor can provide valuable information on the biosynthesis pathway activity through the monitoring of product formation (Fig. 3). It enables to probe the change of pathway activity triggered by the differentiation of gene expression levels. The biosensor approach can be utilised for other biosynthetic pathways with more complex metabolic networks and involving rationally designed perturbation experiments. Compared with other approaches, the fluorescence-based biosensor technique enables relatively easy quantification of substrate, metabolic intermediate or product change intracellularly and extracellularly under in vivo conditions.

Moreover, this study shows that produced mannitol is excessively excreted into the culture medium (Supplementary Table 7). When 2.73 mg/L/OD or more mannitol is synthesised, at least 80% of mannitol is excreted. This further increases to over 95% at higher concentrations of mannitol. C. necator H16 is well known to release low-molecular-weight metabolites such as pyruvate (Raberg et al., 2014) or butanediol (Gascocyte et al., 2021), but not disaccharide trehalose (Løwe et al., 2021). As recently shown, trehalose was not released from salt-stressed C. necator H16 and the secretion was only achieved when the sugar efflux transporter setA from E. coli was expressed (Løwe et al., 2021). However, mannitol is a smaller molecule than the trehalose and appears not to require a specific transporter for extracellular release. Nonetheless, the mechanism of metabolite excretion has not been well characterised despite its great importance to industrial processes development and research has mainly been limited to amino and non-amino organic acid secretion (Pinu et al., 2018). Similarly, a mannitol-specific transporter has not been identified in C. necator H16. However, it is likely that low-molecular-weight metabolites, including mannitol, can be released due to hypo-osmotic stress when cells are subjected to a high intracellular concentration. This process might also involve activation of large- and small-conductance mechanosensitive channels (Kung et al., 2010), genes of which are present in C. necator H16, i.e. mscL (H16_A3399) and mscS (H16_A3040), mscS2 (H16_B0712), mscS3 (H16_B1233), mscS4 (H16_B1855) and mscS5 (H16_B2568).

Finally, due to the highly efficient G-3-P flow of the CBB cycle demonstrated in this study using the example of mannitol production via fructose 6-phosphate intermediate (Fig. 1A), the C. necator H16 strains developed here can serve as a platform for autotrophic production of hexoses and their derivatives. This work proofs an essential step toward constructing an autotrophic cell factory for the production of sugar derivatives from CO2.

5. Conclusions

D-Mannitol is a natural sugar alcohol used as a food additive, surfactant and in pharmaceutical applications. Increasing demand for bio-based products attracts this compound as one of the top chemical opportunities (E4tech (UK) Ltd for LBNet, 2017). Here we engineered

<table>
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<th>Table 2</th>
<th>Production of mannitol using CO2 as carbon source.</th>
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<tr>
<td>Microorganism</td>
<td>Genes introduced for mannitol biosynthesis</td>
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<tr>
<td>Synecococcus sp. PCC 7002</td>
<td>mllD from E. coli and mlp from E. tenella</td>
</tr>
<tr>
<td>Synecococcus sp. PCC 7002</td>
<td>m1D/m1p fusion from M. pusilla</td>
</tr>
<tr>
<td>Cupriavidus necator H16</td>
<td>mllD and mlp from E. siliculosus</td>
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*aaverage of yield and productivity monitored from 53.5 h to the 71 h time point of fermentation.*
C. necator H16 to produce mannitol from CO₂ and use the flux directly from the CBB cycle via G-3-P and F-6-P intermediates. The engineered strain achieved more than 48% carbon efficiency and production of 3.9 g/L mannitol. The yield and titre is comparable to those reported for acetoin, 1,3- and 2,3-butanediols (Windhorst and Gescher, 2019; Gascoyne et al., 2021; Bommaraty et al., 2020) in C. necator H16, which, however, were derived from pyruvate through the glycolysis. This is the first report of chemical bioproduction of mannitol via F-6-P intermediate using CO₂ as sole carbon source in C. necator.

Author contributions

N.M. conceptualized the study. E.K.R.H. and N.M. designed the experiments. E.K.R.H., G.S. and N.M. carried out experiments. E.K.R.H. and N.M. wrote the manuscript. E.K.R.H., G.S., N.P.M. and N.M. reviewed and approved the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found at https://doi.org/10.1016/j.ymneb.2022.02.003.

References


Schlegel, H., Gottschalk, G., Von Bartha, R., 1961a. Formation and utilization of poly-


Steinbüchel, A., Schlegel, H., 1991. Physiology and molecular genetics of poly-


Yunus, I.S., et al., 2018. Synthetic metabolic pathways for photobiological conversion of CO₂ into hydrocarbon fuel. Metab. Eng. 49, 201–211.