Engineering of a modular and synthetic phosphoketolase pathway for photosynthetic production of acetone from CO₂ in *Synechococcus elongatus* PCC 7942 under light and aerobic condition

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Summary

Capture and conversion of CO₂ to valuable chemicals is intended to answer global challenges on environmental issues, climate change and energy security. Engineered cyanobacteria have been enabled to produce industry-relevant chemicals from CO₂. However, the final products from cyanobacteria have often been mixed with fermented metabolites during dark fermentation. In this study, our engineering of *Synechococcus elongatus* PCC 7942 enabled continuous conversion of CO₂ to volatile acetone as sole product. This process occurred during lighted, aerobic culture via both ATP-driven malonyl-CoA synthesis pathway and heterologous phosphoketolase (PHK)-phosphotransacetylase (Pta) pathway. Because of strong correlations between the metabolic pathways of acetate and acetone, supplying the acetyl-CoA directly from CO₂ in the engineered strain, led to sole production of acetone (22.48 mg/L ± 1.00) without changing nutritional constraints, and without an anaerobic shift. Our engineered *S. elongatus* strains, designed for acetone production, could be modified to create biosolar cell factories for sustainable photosynthetic production of acetyl-CoA-derived biochemicals.

Introduction

Concerns about energy security, and environmental issues affecting the sustainable carbon cycle, has focused attention on engineering photosynthetic organisms including plants that might sequester and convert CO₂ to organic materials using solar energy. Genetically modified cyanobacteria have been enabled to produce short-chain alcohols such as ethanol (Deng and Coleman, 1999), isopropanol (Hirokawa et al., 2015; Kusakabe et al., 2013), isobutanol (Atsumi et al., 2009) and 1-butanol (Lan and Liao, 2012; Lan et al., 2013); biochemicals such as ethylene (Ungerer et al., 2012; Xiong et al., 2015), 2-methyl-1-butanol (Shen and Liao, 2012) and 2,3-butanediol (Oliver et al., 2013); and isoprenoids such as limonene (Davies et al., 2014; Kiyota et al., 2014) and α-bisabolene (Davies et al., 2014) by manipulating their metabolic pathways and introducing heterologous pathways. Conversion of CO₂ to biochemicals using cyanobacteria requires production under light and dark conditions. However, neither continuous photosynthetic production of such biochemicals, nor the processes by which they might be separated, has yet been studied extensively.

The nature of the target chemicals is important for the continuous production of CO₂. Highly volatile isoprene is a desirable target chemical because of the instant separation possible during photosynthetic cell growth. Engineered *Synechocystis* sp. PCC 6803 with a chromosomal integration of a heterologous mevalonate pathway has enabled production of isoprene (0.25 mg/g DW; Bentley et al., 2014; Lindberg et al., 2010). In addition, volatile ethylene has been produced from CO₂ within recombinant *Synechocystis* sp. PCC 6803 strains (Shen and Liao, 2012). However, the low enzyme activity of isoprene synthase and the ethylene-forming enzyme are still rate-limiting. In addition to volatility, the target products have to be nontoxic to achieve continuous CO₂ conversion. The production of isobutyraldehyde (less toxic) has been shown to be more suitable than isobutanol production in *Synechococcus elongatus* PCC 7942 (Lan and Liao, 2012). If the target product is highly toxic to cells, an in situ recovery process is required. Plant terpenoids such as limonene (4 mg/L) and α-bisabolene (0.6 mg/L) have been synthetized by engineered *Synechococcus* sp. PCC 7002 in a nitrogen-free medium (Davies et al., 2014). To reduce the toxicity of the products, a simple dodecane overlay was used for in situ extraction.

Acetone has a low boiling point and is a flammable chemical used extensively as a solvent in paints, coatings and adhesives or as an intermediate in the production of polymers. Regarding promising chemicals for continuous CO₂ conversion, acetone is also a good candidate because it is relatively volatile and less toxic...
to cyanobacterial cells. To establish sustainable production of acetone using cyanobacteria, a synthetic pathway based on a Clostridium pathway (converting acetyl-CoA to acetone using acetyl-CoA acetyltransferase, acetoacetyl-CoA transferase and acetoacetate decarboxylase) has been designed and integrated to produce acetone in Synechocystis sp. PCC 6803 (Zhou et al., 2012; Figure 1a). After the engineered strains were cultivated under nutritional limitations (nitrogen and phosphorus free), metabolic shifting under light and aerobic condition to dark and anoxic condition achieved production of acetone (36 mg/L) and acetate (40 mg/L) in the medium in a sealed test tube. Similarly, S. elongatus PCC 7942 has been engineered for isopropanol production via the acetone-producing Clostridium pathway combined with NADPH-dependent secondary alcohol dehydrogenase activity (Kusakabe et al., 2013). The engineered S. elongatus strains have required metabolic shifting for the production of isopropanol (26.5 mg/L) and acetate (150 mg/L). Further optimizations by dark fermentation and anaerobic conditions to light and aerobic condition have lead the enhanced production of isopropanol (146 mg/L; Hirokawa et al., 2015). However, none of strains has produced acetone under light and aerobic condition without a metabolic shift.

Herein, we report engineered S. elongatus PCC 7942 that produced sole acetone from continuous feeding of CO2 under light and aerobic conditions. The product was simultaneously separated from the culture medium. Photosynthetic acetone production was approached in a different way than in previous studies, by replenishing intracellular acetyl-CoA directly from CO2 via ATP-driven carbon flux and a phosphoketolase (PHK)-phosphotransacetylase (Pta) pathway. Finally, we determined that synthetic pathway design for increase the acetyl-CoA supply is the most critical factor for real photosynthetic production of acetone and demonstrated a sustainable photosynthetic platform for the production of acetyl-CoA-derived biochemicals.

Results and discussion

Analysis of acetone-producing S. elongatus PCC 7942 in the presence of acetate

The first generation strains of S. elongatus PCC 7942 used for photosynthetic acetone production were constructed (Table 1; Figure 2), resulting SeAAD (S. elongatus NSI::Bb1s-atoB-atoDA-adc) and SeACD (S. elongatus NSI::Bb1s-atoB-ctfAB-adc) expressing heterologous Escherichia coli and Clostridium acetobutyricum genes (Figure 1a). The strains showed no production of acetone under 5% CO2 bubbling in constant light as reported previously (Kusakabe et al., 2013; Figure 3a). Acetate can be used as a co-substrate for acetoacetyl-CoA transferase (AtoDA/CtfAB) or as a
Table 1 Bacteria strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Reference</th>
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Figure 2 Scheme of heterologous gene integration into *Synechococcus elongatus* PCC 7942: (a) the acetone production pathway from acetyl-CoA to acetone was integrated into NSI of the genome. (b) Genes encoding for phosphoketolase (PHK) or/and phosphotransacetylase (Pta) were integrated into NSII of the genome. (c) Colony PCR results verifying recombinant *S. elongatus* strains using a pair of Se1-fw/rv and Se2-fw/rv for the NSI and NSII integrations, respectively. The DNA sequences were also correctly verified. The target size of each PCR product for cyanobacterial wild type or mutant: wild type (1.9 kb), SeAAD (8.1 kb), SeACD (8.1 kb), SeNAD (7.9 kb), SeNCD (7.9 kb) at NSI and wild type (2.7 kb), SeNAD-X (7.2 kb), SeNAD-P (5.8 kb) and SeNAD-XP (8.2 kb) at NSII.
substrate to supply Acetyl-CoA catalysed by Acetyl-CoA synthetase (Synppc7942_1352) in the acetone production pathway. Thus, we added 10 mM of potassium acetate to the medium when cells were inoculated. As a result, 222.7 mg/L ± 0.17 and 187.3 mg/L ± 8.85 of total acetone (the sum of acetone measured in the medium and captured in the collection bottle) were produced by the SeAAD and SeACD strains, respectively, over 5 days in the presence of acetate (Figure 3b,c). The SeAAD strain having AtoD showed slightly higher production of acetone than did SeACD in the presence of acetate. Although the highest production level of acetone was reported in this study, we measured decreased levels of acetate in the medium. Both SeAAD and SeACD completely utilized 10 mM acetate in the medium within 5 days. When the acetate was completely depleted, production of acetone by cyanobacterial cells stopped.

To investigate the correlation between acetate consumed and acetone produced, we analysed the acetone production by the SeAAD strain by providing different concentrations of acetate in the culture (5 and 10 mM) at 0 day. The correlation coefficient was significantly high ($R^2 = 0.99$) for acetate consumed with acetone produced (Figure 3d). When we calculated the ratio of acetate consumed and acetone produced, 0.39 (mole of acetone/mole of acetate) was calculated, where the theoretical conversion molar yield for the acetone from acetate is 0.5 (mole of acetone/mole of acetate). Therefore, a strong positive correlation between acetone production and acetate consumption was shown. This suggested that carbon flux from acetyl-CoA to acetone was derived from the acetate given to the SeAAD strain. Thus, inefficient supply of acetyl-CoA level could not drive the subsequent enzymatic reactions for acetone production under constant light and in aerobic culture, in the absence of acetate.

**ATP drives photosynthetic sole acetone production in the absence of acetate**

The AtoB thiolase used in the SeAAD and SeACD strains favours the thiolysis of acetoacetyl-CoA. Recent studies have shown that ATP-driven malonyl-CoA synthesis and the decarboxylation reaction in engineered *S. elongatus* PCC 7942 enabled direct photosynthetic 1-butanol production (29.9 mg/L; Lan and Liao, 2012; Lan et al., 2013). ATP-driving metabolic force was capable of supplying acetoacetyl-CoA from acetyl-CoA. Thus, we constructed the two strains, SeNAD (*S. elongatus* NSI::Bb1s-nphT7-atoDA-adc) and SeNCD (*S. elongatus* NSI::Bb1s-nphT7-ctfAB-adc), for acetone production to having two-step reactions catalysed by native AccABCD acetyl-CoA carboxylase and heterologous NphT7 thiolase/acetoacetyl-CoA synthase (Okamura et al., 2010; Figure 4a).

The engineered strains SeNAD and SeNCD were cultivated under constant light and in aerobic culture. As a result, indeed, the SeNAD and SeNCD strains produced 9.96 mg/L ± 0.54 and 5.16 mg/L ± 1.03 of acetone, respectively (Figure 4b,c). The acetone production of the SeNAD strain was twice as high as that of SeNCD. None of the engineered cyanobacterial strains have shown growth-associated production of acetone under light and in aerobic culture (Hirokawa et al., 2015; Kusakabe et al., 2013; Zhou et al., 2012). This is the first report of direct conversion of CO2 to acetone under aerobic and lighted conditions.

Interestingly, the growth reduction in SeNAD (67%) and SeNCD (59%) was also reflected in the growth of SeAAD and SeACD, although the levels of acetone produced (10 mg/L) by the strain SeNAD were significantly lower than the cytotoxic level (between 10 and 100 g/L; Kusakabe et al., 2013). Introducing energy-consuming ATP-driven pathway could be cellular burden in cells. In addition, we checked whether metabolites other than acetone were produced or not, using HPLC and GC-MS analysis. No acetate was measured in the supernatant of the SeNAD and SeNCD cultures (Figure 4d). Thus, our engineered SeNAD and SeNCD strains were capable of producing sole acetone from CO2 via NphT7-mediated pathway. Furthermore, heterologous expression of acetate-independent acetoacetyl-CoA transferase could be
advantageous with the NphT7-mediated pathway in no acetate given system (Alonso-Gutierrez et al., 2013). Although the resulting strain produced sole acetone in lighted, aerobic culture, the levels of sole acetone were quite low due to the limited acetyl-CoA pool.

Increased acetyl-CoA-pool enhanced acetone production via the phosphoketolase pathway

To enhance acetone production via supplying the acetyl-CoA, the SeNAD strain (best producer from the previous result) was chosen for further engineering with the phosphoketolase pathway (Figure 5a). The phosphoketolase pathway from Aspergillus nidulans (Papini et al., 2012) has contributed to increased levels of polyhydroxybutyrate (Kocharin et al., 2013) and fatty acid ethyl ester (de Jong et al., 2014) by supplying endogenous acetate and acetyl-CoA, respectively. Thus, we constructed a SeNAD-X (S. elongatus NSI::Bb1s-nphT7-atoDA-adc NSII::Bb1s-xpkA) strain that expressed the A. nidulans xpkA gene encoding for a phosphoketolase.

As a result, the SeNAD-X strain produced 7.09 mg/L ± 0.51 acetone, which was 26% lower than the level from SeNAD (Figure 5b). As the PHK pathway has been reported for sugar dissimilation on xyllose by some bacteria (bifidobacteria), xyllose-fermenting yeasts or filamentous fungi, those strains generate acetyl-CoA from acetyl phosphate via either acetate kinase and acetyl-CoA synthetase or Pta. Due to the lack of gene annotation of Pta in S. elongatus PCC 7942, the enhancement of acetone by redirecting carbon flux from xylulose-5-phosphate to acetyl-CoA could be failed. This could explain that acetyl phosphate could be accumulated in the cells, SeNAD-X. Accumulated acetyl phosphate could lead to a lower growth rate of SeNAD-X than SeNAD due to altered status of lysine acetylation, which is related to post-translational protein modification of a critical regulatory role (Mo et al., 2015; Weinert et al., 2013).

Thus, we co-expressed the Bacillus subtilis pta gene encoding for phosphotransacetylase, yielding a NAD-XP (S. elongatus NSI::Bb1s-nphT7-atoDA-adc NSII::Bb1s-xpkA-pta) strain to completely metabolize xylulose-5-phosphate to acetyl-CoA. As a result, the SeNAD-XP strain produced 13.74 mg/L ± 2.39 of acetone (a 1.4-fold increase), compared to the SeNAD strain, in the absence of acetate under aerobic and lighted conditions (Figure 5c). Also, no organic acids, including acetate, were measured in the medium after cultivation of SeNAD-XP (data not shown). Interestingly, the SeNAD-P (S. elongatus NSI::Bb1s-nphT7-atoDA-adc NSII::Bb1s-pta) strain showed no growth without direct evidences. The sole expression of Pta in the SeNAD-P strain led to detrimental cell growth in the absence of acetate, yielding a possibly futile cycle of acetyl-CoA via acyl phosphate and acetate. However, their growth recovered in the presence of 10 mM acetate at 0 day. This rescue could be due to high flux of acetate conversion to acetyl-CoA above the critical levels of acetyl-CoA in a cell. Nonetheless, heterologous gene expression of Pta with the PHK pathway was necessary for cyanobacterial cells to enhance the photosynthetic production of acetone.

To investigate the cellular burden due to excessive consumption of ATP driven by NphT7 with xylulose-5-phosphate by PHK pathway for acetone production, the growth of the best producer SeNAD-XP was compared with the growth of the strain SeAAD-XP and SeACD-XP strains where NphT7 was replaced with AtoB. As a result, the SeAAD-XP and SeACD-XP strains showed the
higher cell growth than the strain SeNAD-XP, but the acetone titres from both strains were significantly decreased (Figure 6). As discussed in previous studies (Lan and Liao, 2012; Lan et al., 2013), additional ATP consumption caused adverse effects in the acetone-producing cells, resulting in reduced biomass formation. However, sole synthetic PHK pathway was not enough to provide the driving forces of carbon flux towards higher acetone production. The trade-off in the cell growth and acetone production using the strain SeNAD-XP could be achieved by optimizing expression of NphT7 using tight gene expression systems.

Supplying the acetyl-CoA via the XpkA-Pta pathway and incorporating an ATP dependent step contributed to increasing photosynthetic acetone production (13.74 mg/L ± 2.39) in S. elongatus PCC 7942 under aerobic and lighted conditions. Direct measurement of intracellular acetyl-CoA could be useful to confirm the altered levels of acetyl-CoA in the acetone-producing cyanobacterial strains. Furthermore, a synthetic nonoxidative glycolysis pathway (Bogorad et al., 2013) using another PHK pathway (converting fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate) could be useful to analyze acetyl-CoA metabolism in cyanobacteria. It might also be applied for the improvement of acetone and acetyl-CoA-derived chemicals.

Continuous conversion of CO2 to sole acetone in a controlled flat photobioreactor

To increase production of photosynthetic acetone by SeNAD-XP, the strain was cultivated in a controlled flat photobioreactor (1.8 L) with continuous 5% CO2 bubbling and constant light conditions (Figure 7a). Using the engineered SeNAD-XP strain, a total of 22.48 mg/L ± 1.00 of acetone was secreted into the liquid phase. The production of acetone was monitored by measuring the OD730 and acetone levels (Figure 5b, 5c). The acetone production was compared to the control strain SeNAD-X under the same conditions. The results showed that the engineered strain SeNAD-XP produced significantly higher acetone levels than the control strain SeNAD-X.

Figure 5 Photosynthetic sole acetone production via the phosphoketolase pathway: (a) a scheme of redirection of carbon flux from xylulose 5-phosphate to the acetone production pathway via the phosphoketolase (PHK) pathway with phosphotransacetylase (Pta). Cyanobacterial growth (OD730) and acetone levels were measured in absence of acetate under constant light and 5% CO2 feeding using the (b) SeNAD-X and (c) SeNAD-XP strains. All data are mean ± SD from triplicate cultures.

Figure 6 Trade-off in the cell growth and acetone production in engineered cyanobacteria. Cyanobacterial growth (OD730) and acetone levels were measured in the absence of acetate under constant light and 5% CO2 feeding using the strains SeAAD-XP (red circle), SeACD-XP (blue triangle) and SeNAD-XP (black square). All data are mean ± SD from triplicate cultures.

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vessel and captured in the collection bottle (Figure 7b). The specific production of acetone in the photobioreactor (1.8 L) was 8.65 mg/L/OD730, and it was increased by 1.9-fold, compared to the specific production in a bottle (100 mL), but a lower growth rate was observed.

The total carbon productivity and carbon partitioning to acetone of the SeNAD-XP strain were calculated. As a result, carbon partitioning to acetone of the SeNAD-XP strain was different from the cyanobacterial culture systems, showing significantly higher carbon partitioning to acetone (21%) in the photobioreactor (Figure 7c). Because there were negative correlations of biomass formation with acetone production, optimization of the cyanobacterial cell culture systems would be necessary with long-term cultivation to increase the productivity level of sole production of acetone. Moreover, development of a recycling system of CO2 in the off-gas would be necessary to achieve 100% conversion of CO2, although this also depends on the size of the photobioreactors used.

Conclusions

Acetyl-CoA, a key molecule for biotechnological applications in microbial metabolism, is used as a substrate for the oxidative TCA cycle, acetate, and ethanol production pathways, glyoxylate shunt or fatty acid biosynthesis (Choi and Lee, 2013; Steen et al., 2010). These acetyl-CoA-consuming pathways compete against the heterologous acetone-producing pathway in S. elongatus. In this study, metabolically engineered S. elongatus PCC 7942 was successfully applied to continuously convert CO2 to sole acetone in lighted, aerobic culture (Figure 8). Rewiring the metabolisms with the PHK pathway replenished the pool of acetyl-CoA that was converted to sole acetone along with the ATP-driven carbon flux. Also, combining this with development of the cyanobacterial strain, bioprocessing for acetone recovery enhanced the production of acetone based on the principle of volatile gas–liquid exchange in a controlled photobioreactor. Thus, improvement of the cyanobacterial strains, combined with bioprocessing, will accelerate the development of biosolar cell factories to harvest volatile acetyl-coA-derived chemicals from CO2.

Experimental procedures

Strains and plasmids

All bacterial strains and plasmids used in this work are listed in Table 1. For cloning, E. coli strains were grown in Lysogeny broth medium (containing per litre: 10 g tryptone, 5 g yeast extract, and 10 g NaCl) at 37 °C, when appropriate, the medium was supplemented with 50 μg/mL kanamycin, 100 μg/mL spectinomycin. S. elongatus PCC 7942 was purchased from the ATCC 33912 and used as the production host for acetone from 5% (v/v) CO2 feeding. The neutral site I (NSI) or II (NSII) targeting vectors (SyneBrick vectors) were constructed by inserting either NSI DNA
sequence or NSII DNA sequence from pSyn_1 into the BglBrick vectors (Lee et al., 2011) as a synthetic platform for gene expression in *S. elongatus* PCC 7942. SyneBrick vectors follow the strategy of the BglBrick cloning method to clone target genes into the vectors at the BglBrick restriction enzymes site (EcolI, BglII, BamHI and Xhol). The *E. coli* atoB (Hanai et al., 2007) and atoDA (Hanai et al., 2007), *C. acetobutylicum* ctFA (Hanai et al., 2007; Zhou et al., 2012) and adc (Hanai et al., 2007; Zhou et al., 2012), *Streptomyces* sp. C190 nhf7 (Lan and Liao, 2012; Okamura et al., 2010), *Asgarpillus nidulans* xpkA (Panagiotou et al., 2008; Zhou et al., 2012) and *Balcius subtilis* pta (Zhou et al., 2012) genes were codon-optimized using Gene Designer 2.0 software (DNA2.0, Menlo Park, CA) and synthesized (Genscript Inc., Piscataway, NJ) for efficient heterologous expression in *S. elongatus* PCC7942. Each target gene for acetone production was cloned into SyneBrick vectors (targeting at NSII), yielding pSe2Bb1k-X, pSe2Bb1k-P and pSe2Bb1k-XP genes were cloned into SyneBrick vectors (targeting at NSI) to construct a pSe1Bb1s-AAD and pSe1Bb1s-ACD. The atoB gene in the pSe1Bb1s-AAD and pSe1Bb1s-ACD was replaced to the nhf7 gene, yielding a pSe1Bb1s-NAD and pSe1Bb1s-NCD, respectively. To enhance the acetyl-CoA availability, additional xpkA or/and pta genes were cloned into SyneBrick vectors (targeting at NSII), yielding pSe2Bb1k-X, pSe2Bb1k-P and pSe2Bb1k-XP.

**Transformation of *S. elongatus***

Transformation of *S. elongatus* PCC 7942 was performed as described previously (Golden et al., 1987). The SyneBrick vectors were transferred for chromosomal integration. Recombinant strains were obtained after transferring colonies to fresh selective plates in order to prevent from chromosome segregation. The strains were confirmed by PCR to verify integration of targets into the chromosome (Figure 2), and the DNA sequences were also correctly verified using a pair of Se1-fw (5'-TCT ACT ACA TCT GCC AAC CCA G-3') and Se-1-rv (5'-AAT CTG AAG ACC CGC CAA GTG T-3') for the NSI and a pair of Se2-fw (5'-ATT GTT GAG GCA GGC AAT CAC G-3') and Se-2-rv (5'-TGT CTA CAC CAG AGA CCA ATG G-3') for the NSII.

**Cyanobacterial culture for acetone production**

*Synechococcus elongatus* PCC 7942 and its derivatives (Table 1) were cultivated at 30 °C in the 100 mL culture (Duran bottle with a three-ports cap) under continuous fluorescent light (100 μEm/s) measured by LightScout Quantum meter (3415FSXE; Spectrum, Aurora, IL) in BG-11 medium supplemented with 10 mm MOPS, 5% (v/v) CO₂ gas (monitored by online gas analyser) and 95% (v/v) filtered air were supplied at constant flow rate of 10 cc/min into the medium (Figure 1b). Off-gas line was sequentially connected to two identical collection bottles (500 mL), which contain 500 mL distilled water without headspaces. To collect volatile acetone from either the BG-11 or the off-gas line, a gas-stripping-based recovery systems (Inokuma et al., 2010) were modified (Figure 1b). Ten micrograms per milliliter spectinomycin or/and 10 μg/mL kanamycin were supplemented for selection pressure. One milliliter of IPTG was supplemented into the culture medium at 24 h after inoculation.

**Quantification of acetate, alcohols and acetone**

One milliliter of cyanobacterial cell supernatant or sample from collection bottle was filtered with syringe filter (pore size of 0.2 μm) after centrifugation at 10 000 g for 10 min. For quantification of acetone, ethanol and isopropanol, the samples were analysed by gas chromatography (Model 6890; Agilent Technologies, Santa Clara, CA) equipped with a HP-INNOWAX polyethylene glycol column (30 m × 0.25 mm × 0.25 m) and a flame ionization detector (FID) under the following conditions: oven temperature, from 50 to 240 °C at a rate of 10 °C/min; injector temperature, 250 °C; detector temperature, 250 °C; carrier gas (He); flow rate, 25 mL/min; and split ratio of 1 : 10. For the measurement of the acetate, culture supernatant was passed through a syringe filter after centrifugation at 10 000 g for 10 min. The concentrations of acetate were determined by high-performance liquid chromatography (HPLC system Agilent 1260, Waldbronn, Germany) equipped with a refractive index detector (RID) and an Aminex HPX-87 H Ion Exclusion Column (300 mm by 7.8 mm; Bio-Rad, Hercules, CA) under the following conditions: sample volume of 20 μL, mobile phase of 5 mm H₂SO₄, flow rate of 0.6 mL/min and column temperature of 65 °C.

**Controlled cyanobacterial culture in a flat-panel photobioreactor**

For controlled cyanobacterial culture, a flat-panel photobioreactor (Labfors 5 Lux-LED flat-panel option [637 mm (L) × 298 mm (W) × 79 mm (D); INFOR-HT, Bottmingen, Switzerland) was used to improve production of acetone. Cyanobacterial cells in exponential phase were centrifuged and diluted to OD₇₅₀ of 0.5 in 1.8 L of BG-11 medium. The flat-panel vessel was illuminated under condition of continuous light (100 μE/m²/s), and 30 °C. 5% CO₂ (v/v) was supplied into the cell medium at flow rate of 140 mL/min (0.08 wvm). 3 L NaOH was used to adjust pH 7 in BG-11. Off-gas line was connected to two 500-mL collection bottles that contain 500 mL distilled water for the measurement of total acetone production. For the production of acetone, 1 μm IPTG was added at 24 h after inoculation.

**Calculations for total carbon productivity**

Total carbon productivity was calculated from the previous calculations (Oliver and Atsumi, 2015). Cyanobacterial biomass was calculated based on the measurement of optical density (the conversion factor, 0.22 g DW/L/OD₇₅₀. The carbon mole was calculated from the carbon contents (51.34% of cell biomass using the elemental composition) in a period, divided by 12 g/mole of carbon (Shastri and Morgan, 2005). To calculate total carbon productivity, carbon in acetone (mmol/L) was also calculated from the concentration of total acetone in a period by multiplied by 3 (carbon mole of Acetone C₃H₆O/ per CO₂ fixation). Partitioning of acetone is calculated by carbon productivity of acetone dived by total carbon productivity.

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