Directed Reaction Engineering Boosts Succinate Formation of *Synechocystis* sp. PCC 6803_Δsll1625

Magdalena Mock, Andreas Schmid, and Katja Bühler*

It is known that *Synechocystis* sp. PCC 6803 carrying a partial deletion of the succinate dehydrogenase (*Synechocystis_Δsll1625*) secretes succinate during aerobic cultivation with continuous illumination and in the presence of CO₂. Maximal succinate titers of 2 mM (236 mg L⁻¹) are reported. CO₂ is identified as a crucial parameter for product formation, however, a detailed characterization of different cultivation conditions is still missing. Here the focus is on further reaction engineering to improve the photoautotrophic production of succinate using *Synechocystis_Δsll1625*. Therefore the impact of light availability, illumination regimes, nutrient availability, and external pH on product formation are investigated. Results obtained in this study reveal the importance of these parameters on the formation of succinate and cultivation with light/dark cycles increases the succinate concentration to 3 mM (354 mg L⁻¹) after 28 days of cultivation. Furthermore, cultivation in unbuffered medium under ambient CO₂ conditions even doubled the final succinate titer to 4 mM (472 mg L⁻¹) after 28 days. Taking biomass concentrations into account, a maximal yield of succinate on biomass of 215 mgSucc gCDW⁻¹ is achieved, which is the highest so far reported for the production of succinate utilizing *Synechocystis* as host organism.

1. Introduction

Succinic acid was selected as one of the top 12 building block chemicals from biomass by the US Department of Energy. [1] It is a C₄-dicarboxylic acid and can be used as a precursor for numerous chemicals such as 1,4-butanediol or tetrahydrofuran. [2] In addition, it has great potential for the production of bio-based polymers like polybutylene succinate. [3] As many of these valuable derivatives were produced directly from petroleum-based chemicals, the current demand for succinic acid of 30 000–50 000 tons per year is rather low. [4] However, the bio-based production of succinic acid has improved during the last years, and the market for succinic acid is expected to increase to more than 70 000 tons per year. [5]

As succinate is an intermediate of the tricarboxylic acid (TCA) cycle, a wide range of different microorganisms including natural overproducers as well as recombinant production strains was, and is studied to replace the petrochemical process by a biotechnological approach. Currently, four companies (Reverdia, Succinity, BioAmber, and Myriant) have commercialized the production of bio-based succinic acid. However, the large-scale production of bio-succinic acid is based on common feedstocks like sugars, starch, and molasses and these organic carbon-based feedstocks often turn out to be the main cost driver in such bioprocesses. [4,5] In addition, despite being a renewable carbon source, the sustainability of such processes remains questionable. [6]

Thus, the use of CO₂, as a cheap, and especially abundant inorganic carbon source moved more and more into the focus of biotech research. In this context cyanobacteria are highly interesting organisms. They are prokaryotes performing an oxygenic photosynthesis utilizing water as electron donor. A number of promising products synthesized by cyanobacteria from CO₂ have been reported in the recent years and it is widely accepted that cyano-bacteria are versatile biocatalysts. [7–9] Amongst these organisms, *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) and *Synechococcus elongatus* PCC 7942 (hereafter *Synechococcus*) species are the most prominent examples and the photoautotrophic production of succinate was already demonstrated for both strains. The studies for *Synechococcus* focused on the oxidative branch of the TCA cycle and a final succinate titer of 430 mg L⁻¹ was reported. [10] So far the production of succinate using *Synechocystis* focused on the reductive arm of the TCA cycle as *Synechocystis* is known to secrete succinate in the absence of sugars by fermentation under dark anaerobic conditions. [11] An additional overexpression of the gene coding for the phosphoenolpyruvate carboxylase and a cultivation temperature of 37 °C resulted in the highest reported titer of 1.8 g L⁻¹ (15 mM) under fermentative conditions but at an extraordinary high cell density of 25 gCDW L⁻¹ [12] All those studies have in common that they usually apply one standard condition for their reaction systems without looking at the impact of the various reaction parameters on productivities.

Dr. M. Mock, Prof. A. Schmid, Prof. K. Bühler
Department Solar Materials
Helmholtz Centre for Environmental Research—UFZ
Leipzig 04318, Germany
E-mail: katja.buehler@ufz.de

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ria can only be observed at low cell densities below \( \approx 0.2 \, \text{g}_{\text{CDW}} \, \text{L}^{-1} \) before the culture enters a light-limited stage indicated by a linear growth behavior. This depends on the path length of the light through the culture and incident illumination intensity.\[14\] Here, we investigated the influence of light availability on the photoautotrophic formation of succinate. Succinate-producing \textit{Synechocystis} \textit{Δsll1625} was cultivated at 2% CO\(_2\) in shake flasks and light intensities were stepwise increased. Thereby the light regime described by David et al. for \textit{Synechocystis} sp. PCC 6803 was applied, starting from 25 \( \mu\text{E m}^{-2} \text{s}^{-1} \), and increasing the light intensity up to 200 \( \mu\text{E m}^{-2} \text{s}^{-1} \).\[15\] Results were compared to standard cultivation conditions applying constant light intensities of 50 \( \mu\text{E m}^{-2} \text{s}^{-1} \) and product titers. Essentially the same values of 2.6 \( \text{g}_{\text{CDW}} \, \text{L}^{-1} \) and 2 \( \text{mM} \) of succinate were reached independent of the applied light regime, with most of the succinate being formed during linear and stationary phases. At 25 \( \mu\text{E m}^{-2} \text{s}^{-1} \) growth was clearly light limited and the exponential growth rate decreased from 0.067 to 0.040 \( \text{h}^{-1} \), while succinate production started with a corresponding time shift of 3 days compared to incubation at 50 \( \mu\text{E m}^{-2} \text{s}^{-1} \).

### 2.2. Dark/Light Cycles Promote Succinate Production

Many studies on the production of value-added compounds using photoautotrophic organisms are performed with a continuous and constant illumination of the respective culture. However, in nature, cells have to cope with seasonal changes in light availability, and with the diurnal rhythm of day and night. Thus \textit{Synechocystis} \textit{Δsll1625} was cultivated in light/dark cycles with 16 h of continuous illumination (50 \( \mu\text{E m}^{-2} \text{s}^{-1} \)) and 8 h of complete darkness, representing an average summer day in northern Europe. Switches between both phases were instantaneous and gradual illumination and dimming as typical for sunrise and sunset were neglected. Sampling during this experiment was always done directly before turning off, or turning on the light.

As shown in Figure 3A growth was diminished under these conditions as only a final biomass concentration of 1.8 \( \text{g}_{\text{CDW}} \, \text{L}^{-1} \) (\( \text{OD}_{750} = 9 \)) instead of 2.6 \( \text{g}_{\text{CDW}} \, \text{L}^{-1} \) (\( \text{OD}_{750} = 13 \)) was achieved. In addition, OD values only increased during periods of illumination and stayed constant or even decreased in the darkness. As already observed in reference \[13\], product formation mainly occurred in the stationary phase, where cells do not divide but are still metabolically active. Furthermore, results showed that succinate was also formed without light and the final product concentration was increased from 2 to 3 \( \text{mM} \) and consequently also the yield of succinate on biomass of 197 mg\text{succ} \( \text{g}_{\text{CDW}} \, \text{L}^{-1} \) was higher (continuous illumination: 91 mg\text{succ} \( \text{g}_{\text{CDW}} \, \text{L}^{-1} \)).

The results indicated a positive effect of phases without illumination on the formation of succinate. Therefore the experiment was repeated with altered light/dark periods (Figure 3B). Periods of illumination were reduced to 8 \( \text{h} \) and phases of darkness were prolonged to 16 \( \text{h} \), representing an average day in winter in northern Europe. However, growth was further diminished under these conditions, and only a final biomass concentration of 1.26 \( \text{g}_{\text{CDW}} \, \text{L}^{-1} \) (\( \text{OD}_{750} = 6 \)) was reached. In addition, the

![Scheme of the central carbon metabolism and different routes of the TCA cycle in Synechocystis](image1)

**Figure 1.** Scheme of the central carbon metabolism and different routes of the TCA cycle in \textit{Synechocystis}. The \( \alpha \)-ketoglutarate dehydrogenase for the conversion of \( \alpha \)-ketoglutarate to succinyl-CoA is missing in \textit{Synechocystis} (dotted, grey arrow) but the TCA cycle is closed by an \( \alpha \)-ketoglutarate decarboxylase, succinic-semialdehyde dehydrogenase (pink arrows), and the GABA shunt (blue arrows). The succinate dehydrogenase (SDH) catalyzes the conversion of succinate to fumarate and sll1625 encoding subunit B of the SDH is deleted in the strain used in this study (\textit{Synechocystis} \textit{Δsll1625}). AcCoA, acetyl coenzyme A; GABA, \( \gamma \)-aminobutyric acid; GLU, glutamate; aKG, \( \alpha \)-ketoglutarate.

In our study we utilize the mutant strain \textit{Synechocystis} \textit{Δsll1625} carrying a deletion of sll1625 encoding subunit B of the succinate dehydrogenase (Figure 1). This strain is able to photoautotrophically produce succinate up to 420 mg\text{L}^{-1} (3.5 mM) via the oxidative route of the TCA cycle.\[13\] Here, we focused on the systematic investigation of reaction parameters, and their impact on the photoautotrophic production of succinate, which can be doubled by simply changing the cultivation conditions.

### 2. Results

#### 2.1. Under Continuous Illumination Increasing Light Intensities Do Not Impact Final Product Titers

For the cultivation of photoautotrophic organisms light is the central energy source. In general, exponential growth of cyanobacte-
Figure 2. Cultivation of *Synechocystis* Δsll1625 with increasing light intensity under an enriched CO₂ (2%) atmosphere in shake flasks. ■/▲ represent the growth and ▲/▲ represent the succinate concentration in culture supernatant. Black symbols correspond to increasing light intensities, grey symbols to constant illumination of 50 µE m⁻² s⁻¹. Mean values and standard deviation of biological duplicates are shown.

Figure 3. Cultivation of *Synechocystis* Δsll1625 with light/dark cycles and an enriched CO₂ (2%) atmosphere in shake flasks. A) Each day of cultivation was divided in 16 h continuous illumination at 50 µE m⁻² s⁻¹ and 8 h of complete darkness. B) Each day of cultivation was divided in 8 h continuous illumination at 50 µE m⁻² s⁻¹ and 16 h of complete darkness. ■ represents the growth curve and ▲ represents the succinate concentration in culture supernatant. Mean values and standard deviation of biological duplicates are shown.

succinate titer was decreased to 2 mM, but the biomass specific succinate yield was essentially the same, and reached a value of 188 mgsucc /gCDW⁻¹.

In summary, it was shown that periods of darkness can have a positive impact on the photoautotrophic formation of succinate but the ratio of dark to illuminated periods plays a crucial role.

2.3. The External pH Influences the Formation of Succinate

The use of buffered YBG11 medium was established as cyanobacteria alkalize their growth medium due to the consumption of HCO₃⁻ leading to the liberation of OH⁻ and thus in an increase in pH.[10] For *Synechococcus* it was already shown that an adjustment of the pH to a value of 7.5 reduces the formation of succinate and that titers increase with increasing pH.[10] The underlying mechanism of this effect is not known. To check the influence of the external pH on the succinate formation in *Synechocystis* Δsll1625, shake flask experiments have been carried out in unbuffered YBG11 under constant illumination at 50 µE m⁻² s⁻¹. The initial pH was adjusted to 7.2 (as for the buffered version). It has to be mentioned that the use of unbuffered medium was only possible under ambient CO₂ conditions. Elevated levels
of CO₂ resulted in a significant decrease of pH to a value below 6, which completely prevented growth of *Synechocystis* (data not shown).

The pH value was determined during the cultivation and compared to the pH of a culture grown in parallel in YBG11 medium that was buffered with 50 mM HEPES (Figure 4A). In the unbuffered medium, pH increased to a value of 11 during the first 3 days of cultivation, stayed constant for the following 5 days, and afterward decreased again to settle at a level of about 8.6. Growth rates of *Synechocystis* ∆sll1625 were comparable to the cultivation in buffered medium, only the final biomass concentration was slightly increased to 2.2 g CDW L⁻¹ (OD₇₅₀ = 11 for the unbuffered medium instead of 9), and did not decrease after 12 days of cultivation (Figure 4B). Furthermore, analogous to the results published for *Synechococcus*, succinate accumulation was higher compared to the cultivation with a constant pH, and the final titer was increased by a factor of nearly 5 to about 4 mM.

### 2.4. Higher Salt Concentrations Increase Biomass and Succinate Concentrations

Besides light and CO₂, other nutrients/salts in the media can also limit biomass and product formation due to a restricted availability. To evaluate possible limitations due to medium composition, *Synechocystis* ∆sll1625 was cultivated in 5xYBG11, with a constant illumination of 50 µE m⁻² s⁻¹, and 2% of CO₂ (Figure 5). Cells grew with a rate of 0.083 h⁻¹, which is higher compared to the cultivation in normal YBG11 medium where a growth rate of only 0.067 h⁻¹ was reached. Linear growth started already on the second day of the cultivation in 5xYBG11, most likely because the higher cell densities resulted in an earlier light limitation. Nevertheless, the maximal OD₇₅₀ was nearly tripled, and *Synechocystis* ∆sll1625 grew to a final OD₇₅₀ of 40 which corresponds to a biomass concentration of 8 g CDW L⁻¹. Also the succinate titer was doubled to 4 mM but taking the high biomass concentration into account, the resulting yield of succinate on biomass was lowered from 91 to 59 mg Succ g⁻¹ CDW⁻¹ compared to the cultivation in normal YBG11 medium. Interestingly, when combining 5xYBG11 with unbuffered conditions or light/dark cycles, the culture barely grew and cells turned yellowish after a short period of time (data not shown).
3.1. Light is a Critical Factor during the Cultivation of *Synechocystis*

Light is one of the key substrates for photoautotrophic organisms. While growth of heterotrophic organisms in batch cultivations is divided into a log, an exponential, and a stationary growth phase, phototrophic organisms exhibit an additional linear growth phase subsequent to the exponential phase. This phase is most likely due to a limitation of light as a result of self-shading. A stepwise increase of the light intensity during cultivation was shown to prolong exponential growth and also to increase the product titers in case of the photoautotrophic production of propylene glycol with *Synechocystis*.[14,15] In that case the formation of propylene glycol was shown to be linked to the degradation of the intracellular storage compound glycogen, which was positively affected by increased light intensities.[15] However, succinate excretion by *Synechocystis* ∆sll1625 is not connected to glycogen titers, and perhaps this is the reason why such a correlation was not observed in the case of succinate.[13] In addition, final OD and product titers were essentially the same as for the cultivation with continuous illumination pointing to another limitation than light in this case.

In contrast to light intensities, the cultivation under a day/night regime had a significant impact on the performance of *Synechocystis* ∆sll1625. A cycle of 16 h of constant illumination and 8 h of darkness resulted in diminished growth but increased succinate formation to a yield of 197 mg Succ g<sub>CDW</sub>−1. OD<sub>750</sub> values always decreased a little during periods of darkness while succinate was still produced. This indicates glycogen conversion in the dark periods, which is connected to a reduction in cell volumes, and thus a decrease in the OD values.[13] Thus, succinate formation during periods of darkness seemed to be linked to glycogen turnover while this is not the case when cells are illuminated. However, extended periods of darkness did not have a positive impact on biocatalyst performance, rather the opposite was observed. Possibly, if illumination periods are too short, the amount of glycogen that can be generated as a storage compound for dark metabolism is too low to maintain the cells.

3.2. Cultivation Medium Influences Succinate Titers

Apart from light and CO<sub>2</sub>, pH turned out to be a relevant parameter to increase extracellular succinate concentrations.[13] Cultivation in unbuffered medium boosted succinate titers by a factor of 5. As the final biomass concentration did not change significantly, the biomass specific succinate yield of 215 mg Succ g<sub>biomass</sub>−1 was the highest achieved with *Synechocystis* ∆sll1625 (Figure 6). A positive effect of this approach was already published for the photoautotrophic succinate production via the oxidative route of the TCA cycle with *Synechococcus*.[10] For *Synechococcus*, no succinate transporter was annotated. Thus Lan and Wei used BLAST for seven of the succinate transporters described for *Escherichia coli* against the genome of *Synechococcus*.[10] The BLAST was conducted on the amino acid level and resulted in one possible hit with an identity of about 30%. The corresponding protein DauA belongs to the superfAMILY of sulfate transporters and was recently identified to be involved in the succinate import of *E. coli*.[16] Thereby a pH-dependent activity was shown, as DauA...
played a more important role in succinate uptake at alkaline pH.\[^{[16]}\] For \textit{Synechococcus}, it was hypothesized that this pH dependency might result in an increased export of succinate at higher pH. However, experimental evidence is still missing. Also for \textit{Synechocystis} the presence of a specific succinate transporter is unknown. Therefore, the protein BLAST of the seven transporters from \textit{E. coli} was repeated for \textit{Synechocystis}. Similar to \textit{Synechococcus} only \textit{DauA} gave four possible hits. One of the possible ORFs codes for BicA, which is a bicarbonate transporter, and can thus be excluded as a possible succinate transporter. The other three ORFs (\textit{slr0096}, \textit{slr1229}, and \textit{slr1776}, GeneBank accession numbers: AGF52860.1, AGF51203.1, AGF50474.1) had a query cover of 79% or higher, and code for proteins of the sulfate-transporter superfamily and therefore might be identical to \textit{DauA}. Thus, the hypothesis from Lan and Wei could also explain the results obtained for \textit{Synechocystis} in this study.

In addition to the pH, the availability of nutrients in the culture medium can also influence biocatalytic performance of \textit{Synechocystis}. For example, it was stated in the literature that the phototrophic formation of ethylene is increased during cultivation in the presence of higher nutrient concentrations.\[^{[17]}\] When using 5xYBG11, the biomass concentrations, as well as the final succinate titer, were increased, and \textit{Synechocystis}, \textit{Δslr1625} reached a final succinate concentration of 4 mM (472 mg L\(^{-1}\)). Nevertheless, taking the high biomass concentration into account, the resulting yield on biomass is lower compared to the cultivation in unbuffered medium or the cultivation with light/dark cycles (Figure 6). Unfortunately, \textit{Synechocystis}, \textit{Δslr1625} did not grow in 5xYBG11 medium without the addition of HEPES buffer to investigate the impact of combined optimized cultivation conditions.

### 3.3. Cyanobacterial Routes toward Succinate

In 2015 the first publication on the production of succinate utilizing \textit{Synechocystis} as host organism was published\[^{[11]}\] and it was shown that \textit{Synechocystis} excretes succinate under dark and anaerobic conditions via the reductive arm of the TCA cycle. This approach was taken up in several other studies and by metabolic engineering and adaptation of cultivation conditions a final succinate titer of 1.8 g L\(^{-1}\) (15 mM) could be achieved, which is the highest titer reported so far for the phototrophic production of succinate.\[^{[12,18]}\] However, taking the high biomass concentration of 25 BCDW L\(^{-1}\) into account, the approach via the reductive branch of the TCA cycle resulted in a yield of 72 mg Succ BCDW\(^{-1}\).\[^{[12]}\]

As an alternative to \textit{Synechocystis}, \textit{Synechococcus} was also used to produce succinate from CO\(_2\). In contrast to the studies on \textit{Synechocystis}, the oxidative branch of the TCA cycle was utilized in \textit{Synechococcus}, and a maximal biomass specific succinate yield of 843 mg Succ BCDW\(^{-1}\) is reported.\[^{[10]}\]

A comparison of the results obtained for \textit{Synechocystis} under dark and anaerobic conditions with the results obtained in this study clearly shows that the yields of succinate on biomass are higher if succinate is produced via the oxidative route of the TCA cycle although cultivation times are longer. However, the published examples include two steps of cultivation. Biomass is formed in periods of illumination and afterward cells are harvested and concentrated for cultivation under dark and anaerobic conditions. Taking this into account, differences become minor, and the labor input is lower if the oxidative branch is utilized. A comparison with the results published from Lan and Wei using \textit{Synechococcus} as host organism shows the potential of the phototrophic production of succinate. Despite being interesting microbial cell factories for biotechnological processes, there are still major bottlenecks to overcome before cyanobacteria will be applied for truly competitive production processes. Especially when considering bulk chemicals like succinate where established production routes are already in place, pending challenges appear huge. In the case of succinate a couple of biotechnological processes have been developed based on heterotrophic organisms and productivities of up to 3 g L\(^{-1}\) h\(^{-1}\) have been achieved with the respective strains.\[^{[19]}\]

Compared to those examples, rates and titers which we can reach with \textit{Synechocystis} sp. PCC 6803 are at least factor 1000 too low to reach a competitive level.

Reaction engineering, as performed in this study, illustrates that parameters like light, illumination regimes, and pH have a huge impact on reaction performance and need to be looked at on a case to case basis. Nevertheless, productivities could at best be improved by a factor of five. A better knowledge on the overall cyanobacterial system on all levels is required to enable directed metabolic engineering to enhance formation and secretion of succinate by the whole cell biocatalyst. This includes the interplay of light and dark reactions with storage compound turnover, overall role of the TCA cycle in cyanobacterial metabolism, and the impact of pH on the cellular physiology. Besides this, succinate titers may also be increased by higher biomass concentrations. Typically cell densities are limited to 3 BCDW L\(^{-1}\) in established closed photo-bioreactors like flat panels and tubular systems. One approach to overcome this limitation could be the cultivation of \textit{Synechocystis} as a biofilm as recently reported in ref.\[^{[20]}\]. Thereby, 40 g L\(^{-1}\) were reached with \textit{Synechocystis} sp. PCC 6803. Assuming a succinate yield of \(\approx 1.8\) BCDW\(^{-1}\) as a benchmark, cultivation in a biofilm format would result in a succinate titer of 40 g L\(^{-1}\), and a productivity of 0.08 g L\(^{-1}\) h\(^{-1}\) (assuming a stable production over 20 days), reducing the necessary improvement factor from 1000 to 40.

### 4. Experimental Section

**Chemicals:** All chemicals used in this work were obtained in the highest purity available from Carl Roth GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), Sigma-Aldrich (St. Louis, USA), and Th. Geyer GmbH & Co. KG (Renningen, Germany).

**Cultivation Conditions:** If not stated otherwise, wild type and the \textit{Δslr1625} mutant of the substrain \textit{Synechocystis} sp. PCC 6803_moscow were grown in YBG11 medium containing 50 mM HEPES. For the mutant strain, the medium was supplemented with 50 µg ml\(^{-1}\) kanamycin.\[^{[21]}\]

For solid medium cultivations, BG11 supplemented with 0.75% agar was used.\[^{[22]}\] Liquid pre-cultures were inoculated with \textit{Synechocystis} from an agar plate. Main cultures were inoculated with liquid pre-cultures to an initial OD\(_{730}\) of 0.05. Cultivation was carried out in a Multitron shaker (Infors, Bottmingen, Switzerland), equipped with LED panels at 30 °C, 150 rpm, and 75% relative humidity. Depending on the experimental set-up, illumination was either kept at a constant value of 50 µE m\(^{-2}\) s\(^{-1}\), or was increased stepwise from 25 to 200 µE m\(^{-2}\) s\(^{-1}\), or light/dark cycles were applied. The CO\(_2\) concentration during cultivation was set to 0.04% (ambient conditions) or was increased to 2% or 5%. During
cultivation planktonic cell growth of Synechocystis sp. was followed spectrophotometrically measuring the optical density at a wavelength of 750 nm (OD\textsubscript{750}), ensuring no overlap with chlorophyll absorptions. For the calculation of the CDW from OD\textsubscript{750} values correlation factors of 0.17 for Synechocystis wild type and 0.21 for the ∆1625 mutant were used.\cite{13}

**Determination of Extracellular Succinate Levels:** Succinate was quantified by HPLC on a Dionex Ultimate 3000 system (Thermo Fisher Scientific, Waltham, USA) equipped with a RI detector and a HyperREZ XP Carbohydrate H\textsuperscript{+} column (300 × 7.7 mm, 5 µM). 5 mM H\textsubscript{2}SO\textsubscript{4} was used as eluent with a flow rate of 0.75 mL min\textsuperscript{-1}. The column temperature was set to 25 °C and a volume of 20 µL was injected. Samples were centrifuged for 10 min at rcf 17 000 and the supernatant was transferred to HPLC vials. Quantification was based on calibration curves prepared with standard concentrations of succinic acid.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

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\[1\] T. Werpy, G. Petersen, Top Value Added Chemicals from Biomass: Volume I—Results of Screening for Potential Candidates from Sugars and Synthesis Gas, U.S. Department of Energy, Oak Ridge, TN 2004.
\[2\] I. Bechthold, K. Bretz, S. Kabasci, R. Kopitzky, A. Springer, Chem. Eng. Technol. 2008, 31, 647.
\[3\] S. Choi, C. W. Song, J. H. Shin, S. Y. Lee, Metab. Eng. 2015, 28, 223.
\[4\] M. L. A. Jansen, W. M. van Gulik, Curr. Opin. Biotechnol. 2014, 30, 190.
\[5\] R. G. Mathys, A. Schmid, B. Witholt, Biotechnol. Bioeng. 1999, 64, 459.
\[6\] D. C. Ducat, J. C. Way, P. A. Silver, Trends Biotechnol. 2011, 29, 95.
\[7\] S. A. Angermayr, A. G. Rovira, K. J. Hellingwerf, Trends Biotechnol. 2015, 33, 352.
\[8\] J. W. K. Oliver, S. Atsumi, Photosynth. Res. 2014, 120, 249.
\[9\] P. Savakis, K. J. Hellingwerf, Curr. Opin. Biotechnol. 2015, 33, 8.
\[10\] E. I. Lan, C. T. Wei, Metab. Eng. 2016, 38, 483.
\[11\] T. Osanai, T. Shirai, H. Iijima, Y. Nakaya, M. Okamoto, A. Kondo, M. Y. Hirai, Front. Microbiol. 2015, 6, 1064.
\[12\] T. Hasunuma, M. Matsuda, Y. Kato, C. J. Vavricka, A. Kondo, Metab. Eng. 2018, 48, 109.
\[13\] M. Mock, A. Schmid, K. Buehler, Algal Res. 2019, 43, 101645.
\[14\] R. M. Schuurmans, J. C. P. Matthijs, K. J. Hellingwerf, Photosynth. Res. 2017, 132, 69.
\[15\] C. David, A. Schmid, L. Adrian, A. Wilde, K. Bühler, Biotechnol. Bioeng. 2018, 115, 300.
\[16\] E. Karinou, E. L. R. Compton, M. Morel, A. Javelle, Mol. Microbiol. 2013, 87, 623.
\[17\] J. Ungerer, L. Tao, M. Davis, M. Ghirardi, P.-C. Maness, J. Yu, Energy Environ. Sci. 2012, 5, 8998.
\[18\] T. Hasunuma, M. Matsuda, A. Kondo, Metab. Eng. Commun. 2016, 3, 130.
\[19\] J. H. Ahn, Y. S. Jang, S. Y. Lee, Curr. Opin. Biotechnol. 2016, 42, 54.
\[20\] A. Hoschek, I. Heuschkel, A. Schmid, B. Bühler, R. Karande, K. Bühler, Bioresour. Technol. 2019, 282, 171.
\[21\] S. Shcolnick, Y. Shaked, N. Keren, Biochim. Biophys. Acta 2007, 1767, 814.
\[22\] R. Y. Stanier, R. Kunisawa, M. Mandel, G. Cohen-Bazire, Bacteriol. Rev. 1971, 35, 171.