# *Supplementary Material*

Table S1: **Used oligonucleotides, plasmids and strains.**

| Oligo name | Sequence | Function |
|---|---|---|
| PAH096 | ACAAATTCCCAATTTAGTGGAGGTTACTAGATGAA AAAAACCCAACATCTGG | Construction primer |
| PAH097 | TCGTTTTATTTGATGCCTGGCTGCACTATTTTTCGA ACTGCGGGTGGCTCCAAGCGCTCTGGAATACGAAA CCCTCG | Construction primer |
| BVMO1 | AGCTTTCGCTAAGGATGATTTCTGGTTCCACCAGC AAAATTCG | Construction primer |
| BVMO2 | CCTTGCCCTTTTTTGCCGGACTGCATATAAACGCA GAAAGGCC | Construction primer |
| BVMO 3 | AGCTTTCGCTAAGGATGATTTCTGGGGAAGGGATA GCAAGCTAATTTTTATG | Construction primer |
| BVMO 4 | TAACCTCCACTAACTTCTTGGCGATTGTATC | Construction primer |
| BVMO 5 | ATCGCCAAGAAGTTAGTGGAGGTTACTAGATG | Construction primer |
| sBVMO1 | AAAGGGAATAAGGGCGACAC | Sequencing primer |
| sBVMO2 | GAGCTTTGTCTATTGCTACTCC | Sequencing primer |
| sBVMO3 | AACGCGAAGTAATCTTTTCG | Sequencing primer |

| Plasmid name | Characteristics | Function | Reference |
|---|---|---|---|
| pEERM3_Km | Integrative vector, $Km^R$ | Backbone for pAH059 | (Englund et al. 2015) |

| | | | |
|---|---|---|---|
| pCom10_Capro | pCom10-derived, harbouring *bvmo* gene | Template for BVMO | (Karande et al. 2018) |
| pPMQAK1 | Replicative vector, $Km^R$, $Amp^R$ | Backbone for pAH063 | (Huang et al. 2010) |
| pAH059 | pEERM3_PnrsB_*bvmo* | Integrational shuttle vector | This work |
| pAH063 | pPMQAK1_PnrsB_*bvmo* | Episomal shuttle vector | This work |
| pAH064 | pPMQAK1_PpetE_*bvmo* | Episomal shuttle vector | This work |

| Strain | Characteristics | Reference |
|---|---|---|
| *E. coli* DH5alpha | *supE44*, *ΔlacU169* (*80lacZ*, *ΔM15*) *hsdR17 recA1 endA1 gyrA96 thi*-1 *relA1* | (Hanahan 1983) |
| *Syn*6803 | Wild-type | (Stanier et al. 1971) |
| *Syn*6803_empty | Control strain, *KmR* inserted into neutral site I | This work |
| *Syn*6803_Ni_cBVMO | *Syn*6803, PrnsB_*bvmo*_$T_{double}$ *KmR* inserted in neutral site I | This work |
| *Syn*6803_Ni_pBVMO | *Syn*6803 containing plasmid pAH063 | This work |
| *Syn*6803_Cu_pBVMO | *Syn*6803 containing plasmid pAH064 | This work |

All oligonucleotides were ordered from Eurofins scientific, Luxembourg.

Sequence of *Syn*6803_Ni_pBVMO

Consensus sequence from reads with sBVMO1, sBVMO2 and sBVMO3. *Red, italic – PnrsB promoter*, **Red, bold – RBS***, **blue, bold – BVMO gene sequence**, **black, bold – T$_{double}$ Terminator**.

TTCCTTTTTCATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT
TGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGC
CACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATC
ACGAGGCAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTCTGG*TTC*
*CACCAGCAAAATTCGCATCGCCTCTGCCTTTTTTATAACGGTCTGATCTTAGCGGGGGAAGGA*
*GATTTTCACCTGAATTTCATACCCCCTTTGGCAGACTGGGAAAATCTTGGACAAATTCCCAATT***T**
**AGTGGAGGT**TACTAGATG**AAAAAAACCCAACATCTGGACGCCATCGTCATCGGTGC**
**CGGCTTTGGCGGCATGTACATGCTGAAAAAGCTGCGTGACGAGCAGGGCCTGAAA**
**GTGCGCGTCTTTGACAAGGCCGGCGGCGTGGGCGGCACCTGGTACTGGAACCGTT**
**ACCCCGGCGCGCTCTCCGACACCGAGAGCTTTGTCTATTGCTACTCCTGGGACAAA**
**GAGCTGCTGCAAGAGATGCACATCACCACGCGCTATGTGACGCAGCCGCAAATTCT**
**GTCGTACCTGGAGCATGTGGCCGACCGCCACAACCTGCGCCCCGACATCCAGCTC**
**AACACCGGCATCACCGCCGCCCACTTCAACGAAGCCACCAACCTGTGGGAAGTGA**
**AAACCGACACCGGCGAGGCCTACACCGCCAAATTCCTGGTGACCGCGCTGGGCCT**
**GCTCTCGGCCACCAACGTCCCCAAGATCAAGGGGCTCGACACCTTCCAGGGCGAG**
**TGGCTGCACACCGGCAACTGGCCCGAAGGCGTGCAATACGACGGCAAGCGCGTGG**
**GCGTGATTGGCACGGGCTCCACCGGCACCCAGGTCATCACCGCCATTGCGCCCAA**
**GGTCGAGCACCTGACGGTATTCCAGCGCTCGCCCCAGTACAGCGTGCCCGTGGGC**
**AACGGCCCGGTCACGCCCGAATACGTGGCAGAGGTGAAGAAGAACTACGACGAGA**
**TCTGGGAGCAGGTGAAGGGCTCGGTGGTGGCCTTTGGCTTCAAGGAGAGCACCGT**
**GTCGGCCATGAGCGTGTCGGAAGAAGAGCGCCAGGCCGTGTTCCAGAAAGCCTGG**
**GACAACGGCGGTGGTTTCCGCTTCATGTTCGAGACCTTCTGCGACATCGCCACCGA**
**TGAGCGCGCCAACAAGGCGGCGCAAGACTTCATCCGCAGCAAGATTGCCGAAATC**
**GTCAAGGACCCCGAGACGGCACGCAAGCTCATGCCGCAAGACCTGTACGCCAAGC**
**GCCCGCTGTGCGACAGCGGCTACTACGCCACCTACAACCGGCCCAATGTCGATCTG**
**GTCGATGTCAAGGCCAACCCGATTGTCGAAATCACCCCCAAGGGCGTCAAAACCAC**
**CGATGGCGTGGAGCACGAACTGGACATGCTGATTTTTGCCACTGGCTTTGACGCGG**
**TGGATGGCAACTACACCAAGATCGACATCCGTGGCCGCAATGGCCTGACGATTCAA**
**GAGCAGTGGAAATCAGGCCCTAGCAGCTACATGGGCGTGGCCAATGCCAACTTCC**
**CCAACATGTTCATGGTGCTGGGCCCCAACGGCCCCTTCACCAACCTGCCACCGGCC**
**ATCGAGAGCCAGGTCGAGTGGATTGCCGCGCTGATCAAGGATGTGAACGCCAAGG**
**ACCTGAAAACCGTGGAGGCCACCACCGCCGCCGAAGCCGGCTGGACCAAGACCTG**
**CCAGGACATCGCCAACATGACGCTGTTCCCCAAGGCCGACTCCTGGATTTTTGGCG**
**CAAACATCCCCGGCAAAACCAACACCGTGTACTTCTACATGGCGGGCCTGGGTGCT**
**TACCGGCAAGAGCTGTCGGCGGTGCAAAACAAGGGTTACGAGGGTTTCGTATTCC**
**AG**AGCGCTTGGAGCCACCCGCAGTTCGAAAAATAGTGCAG**CCAGGCATCAAATAAAAC**
**GAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAAC**
**GCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA**T
GCAGTCCGGCAAAAAAGGGCAAG

Equations for dynamic modeling using *Berkeley Madonna* software

```
METHOD RK4

STARTTIME = 0
STOPTIME= 1440
DT = 0.001
DTOUT= 2


{Mass Balances}
S'=F + G - kB - kP        ; Cyclohexanone
B'=kB                     ; Product
P'=kP                     ; Cyclohexanol
F=IF TIME>=t1 then 0 else f1
G=IF TIME>=t2 then 0 else f2

{Initial Conditions}
INIT S=0   ; C-one
INIT B=0   ; C-ol
INIT P=0   ; Product

{Kinetics}
kP=vmax*S/(Ks+B/k1*Ks+S+S*S/k0)*1/(1+P/k2)*X        ; BVMO reaction with inhibition
kB=0.002*S/(7.420+S)*X                                    ; keto reduction

{Constants}
X=1.02                    ;biomass [g L-1]
t1=240
t2=480
f1=0.3*0.032*X            ;C-one feed 1 before 4h, mmol L-1 min-1
f2=0.7*0.032*X            ;C-one feed 2 before 8h, mmol L-1 min-1
vmax=0.091                ; mM min-1
Ks=0.079                  ; mM
k0=5.4                    ;C-one inhibition, mM
k1=0.00237                ;C-ol inhibition, mM

k2=2.0                    ;e-CL inhibition, mM
```

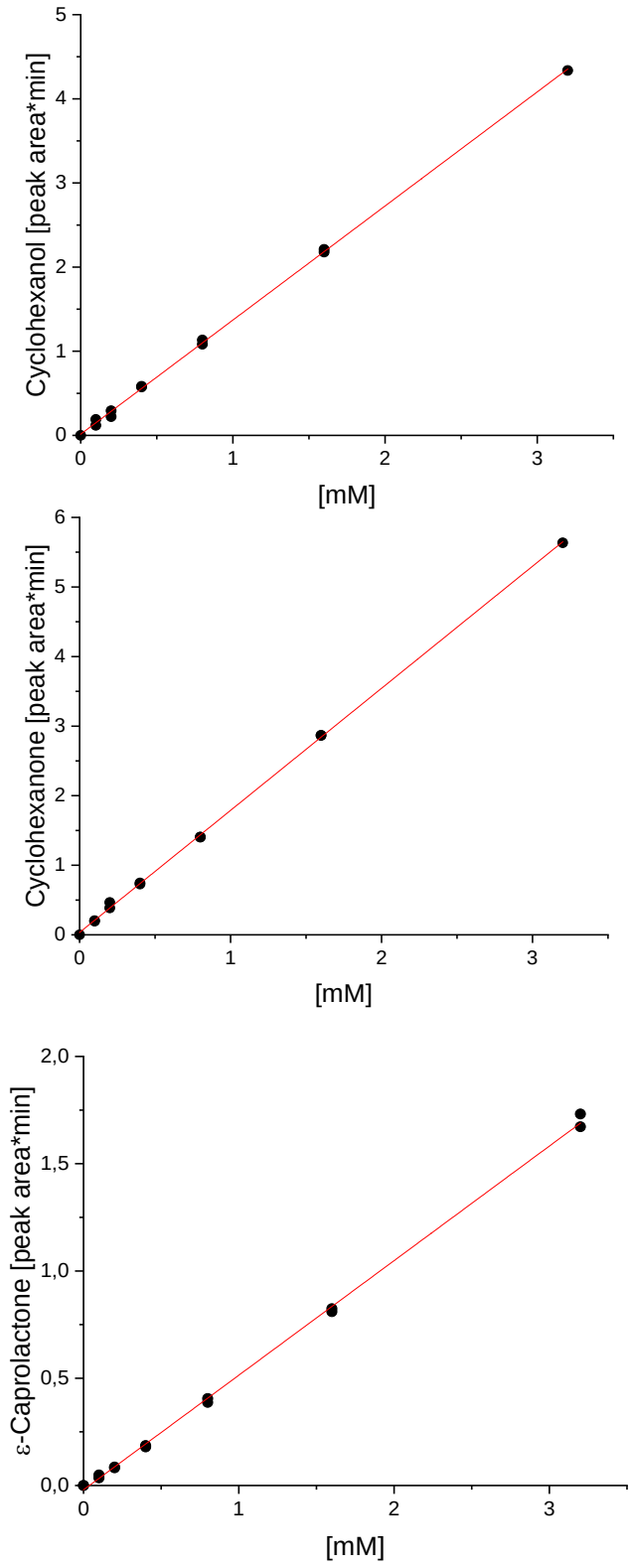


Fig. S1: **Calibration curves for GC-quantification** of C-ol, C-one and ε-Cl. For details, see Material and Method section.

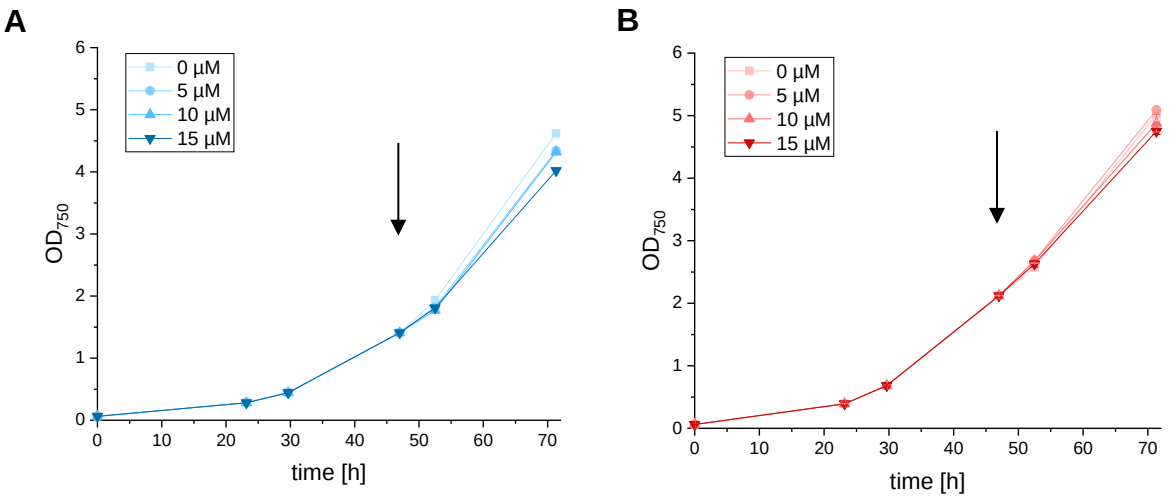


Fig. S2: **Effect of different $Ni^{2+}$ concentrations on growth of *Syn*6803_empty (A) and *Syn*6803_Ni_pBVMO (B).** $Ni^{2+}$ was added after 48h of growth (black arrow). *Syn*6803_empty harbouring a *KmR* inserted into neutral site I was used as a control to differentiate between sole $Ni^{2+}$ effects and (additive) BVMO expression effects. Cells were cultivated at 30 °C, 50 $\mu mol_{photons}$ $m^{-2}$ $s^{-1}$ 2% $CO_2$. Error bars represent SD (n=2).

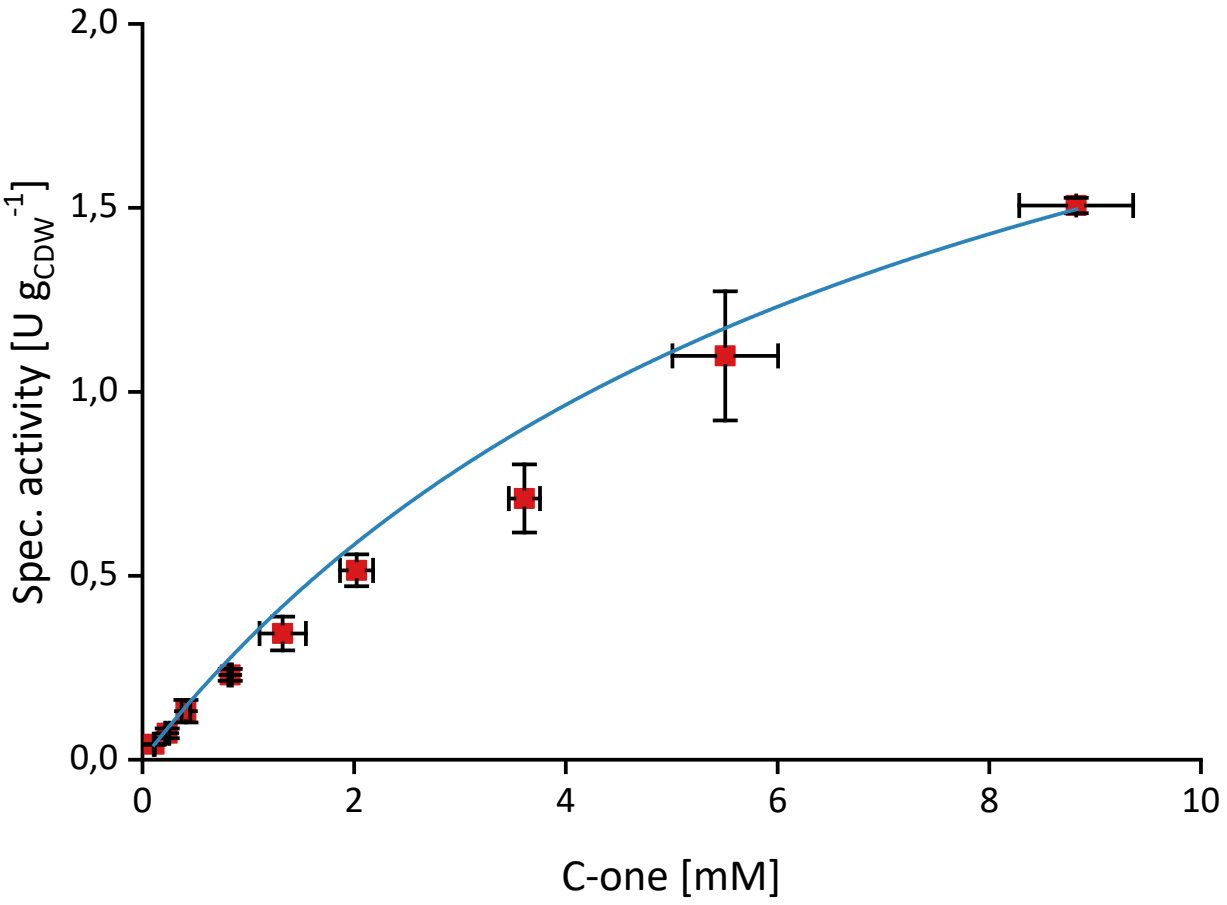


Fig S3: **Characterization of keto reduction in *Syn*6803_wt**. Experiments were conducted as short-term assays with varying C-one concentration. Conditions: 1 $g_{CDW}$ $L^{-1}$, 30min, 0…10 mM cylohexanol. $K_s$=7.4 ± 0.6 mM; $v_{max}$ = 2.75 ± 0.15 U $g_{CDW}^{-1}$

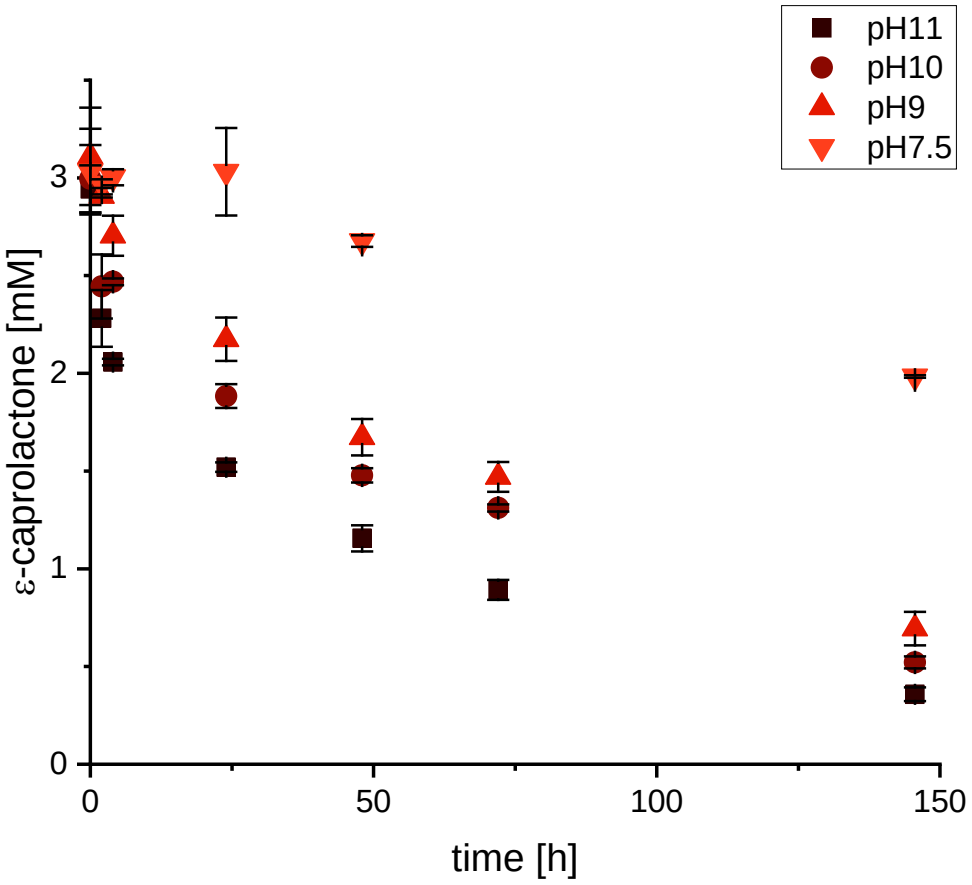


Fig S4: **Abiotic ε-Cl hydrolysis at different pH**. 3 mM ε-Cl was added to YBG11 medium adjusted to the given pH (11, 10, 9 and 7.5, which is the used standard medium), incubated under assay-like conditions and product hydrolysis was followed by lactone quantification via GC.

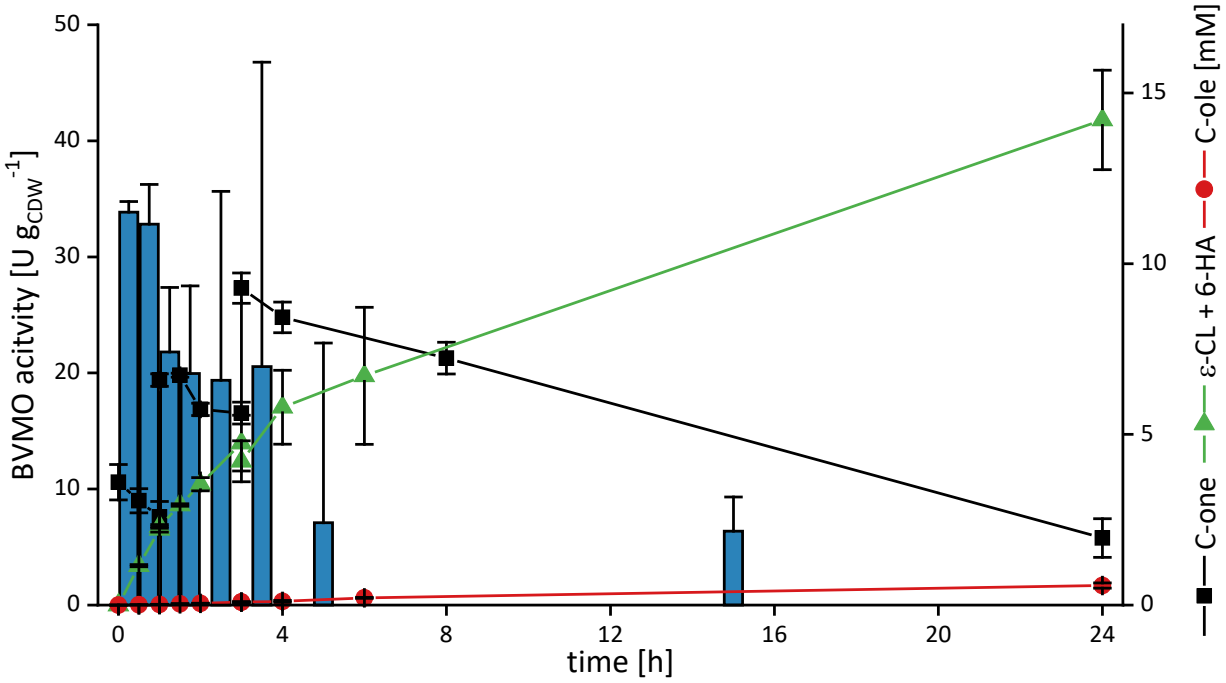


Fig. S5: **Long-term stability of BVMO-reaction in Syn6803_Ni_pBVMO**. Navy bars: BVMO activity, calculated in U (=µmol [ε-Cl + 6-HA] $min^{-1}$) $g_{CDW}^{-1}$; Squares/black line: C-one; Triangles/green line: total product [ε-Cl + 6-HA]; circles/red line: C-ol. Assays were conducted using 20 mL cell suspension in 100 mL screw-capped shaking flasks at standard conditions. 5 mM C-one was fed initially and after 1 and 3 hrs. All data derived from ≥2 biological and ≥2 technical replicates.

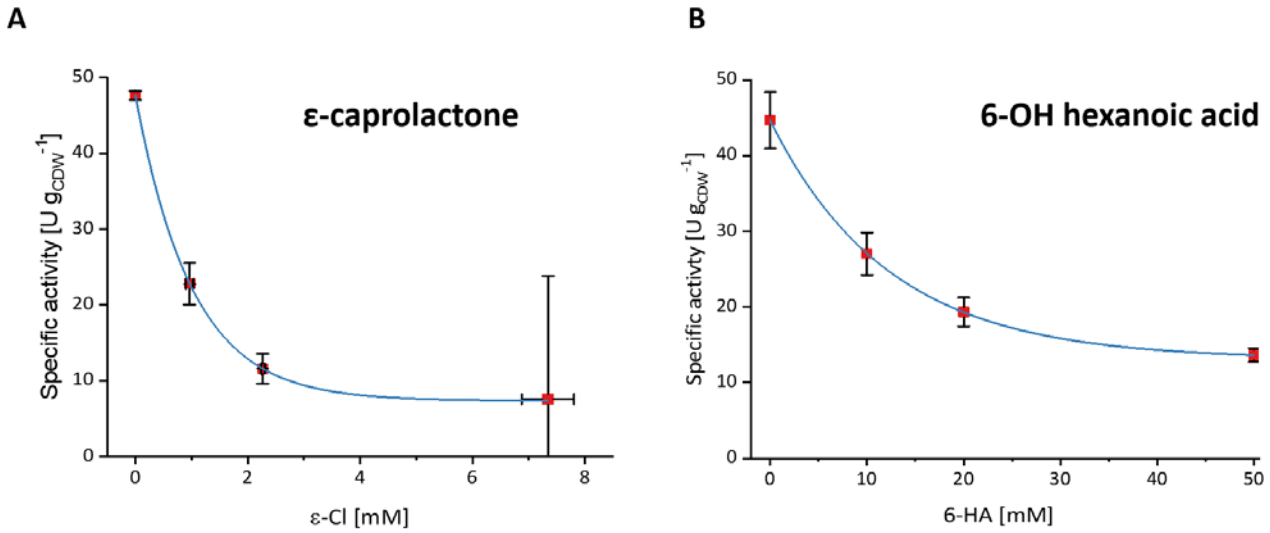


Fig. S6: **Effect of ε-Cl (A) and 6-HA (B) on BVMO activity in Syn6803_Ni_pBVMO.** Product inhibition was tested in whole-cell assays (30min), with defined concentrations of ε-Cl or 6-HA added prior to the assay. BVMO activity, in U (=µmol [ε-Cl + 6-HA] $min^{-1}$) $g_{CDW}^{-1}$ was calculated from the product formed during the assay. All data derived from ≥2 biological and ≥2 technical replicates.

**Table S2:** Comparison of low light and high light conditions for a BVMO biotransformation in a 2-L photo STR **(Infors AG, Bottmingen, Switzerland)**

| **Parameter** | **HL HC** | **LL HC** |
|---|---|---|
| Aeration | | |
| *Air [L $min^{-1}$]* | 2-0.2[a] | 2-0.2[a] |
| $CO_2$ *[mL $min^{-1}$]* | 20 | 20 |
| Light [$\mu mol_{photons}\, m^{-2}\, s^{-1}$] | | |
| for growth | 150-200-250[b] | 100-175-250[b] |
| for biotransformation | 700 | 250 |
| C-one feed [set to $U\, g_{CDW}^{-1}$] | 30 | 30 |
| Biomass conc.[c] [$g_{CDW}\, L^{-1}$] | 1.02 ± 0.01 | 1.00 ± 0.03 |
| Initial vol. productivity [$g\, L^{-1}\, h^{-1}$] | 0.174 ± 0.022 | 0.186 ± 0.002 |

LL – low light, HL – high light, LC – low carbon, HC – high carbon

[a] For biotransformation, Air supply was reduced to 0.2 $min^{-1}$.

[b] Stepwise increase of light intensity during growth.

[c] Biomass concentration at the start of the biotransformation, assessed via $OD_{750}$.

**References:**

Englund, E., J. Andersen-Ranberg, R. Miao, B. Hamberger, and P. Lindberg. 2015. 'Metabolic engineering of Synechocystis sp. PCC 6803 for production of the plant diterpenoid manoyl oxide', *ACS Synth Biol*, 4: 1270-8.

Hanahan, D. 1983. 'Studies on transformation of *Escherichia coli* with plasmids', *J Mol Biol*, 166: 557-80.

Huang, H. H., D. Camsund, P. Lindblad, and T. Heidorn. 2010. 'Design and characterization of molecular tools for a synthetic biology approach towards developing cyanobacterial biotechnology', *Nucleic Acids Res*, 38: 2577-93.

Karande, R., D. Salamanca, A. Schmid, and K. Buehler. 2018. 'Biocatalytic conversion of cycloalkanes to lactones using an in-vivo cascade in *Pseudomonas taiwanensis* VLB120', *Biotechnol Bioeng*, 115: 312-20.

Stanier, R. Y., R. Kunisawa, M. Mandel, and G. Cohen-Bazire. 1971. 'Purification and properties of unicellular blue-green algae (order Chroococcales)', *Bacteriol Rev*, 35: 171-205.