Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

Photosynthetic Production of Nitrogen-Rich Compound Guanidine

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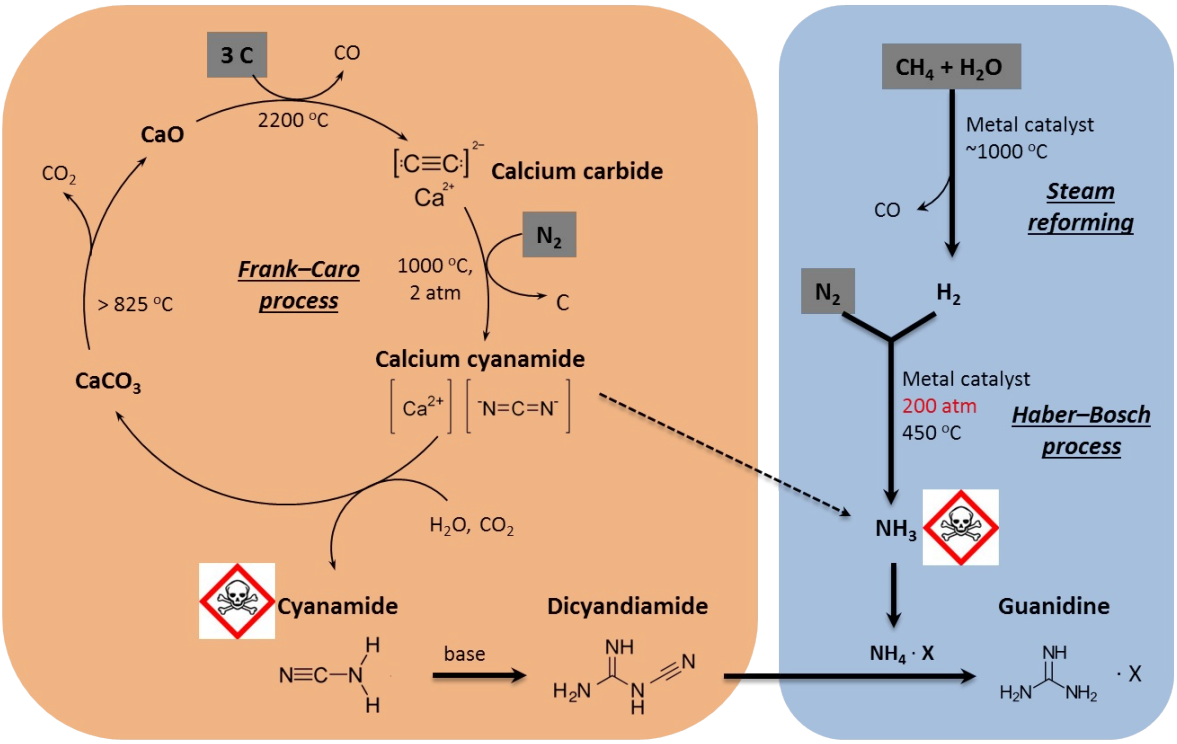
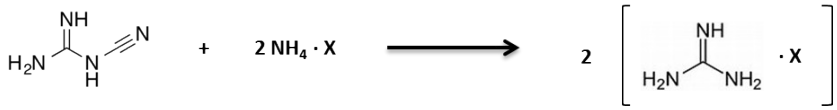
A.



B.



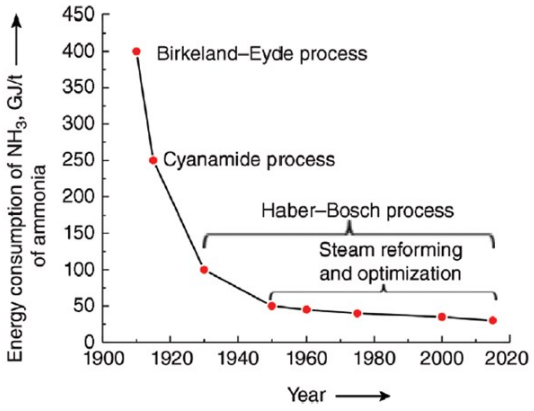


Figure S1 Traditional pathway for guanidine synthesis and the associated energy consumption. (A) Traditional pathway for guanidine synthesis from coal, methane and nitrogen. [1] (B) The energy cost of guanidine synthesis is mostly attributed to the Cyanamide process. Adapted from Patil et al.[2] "X" stands for an anion that may form salt with ammonium. N_2-fixation from the Cyanamide process is about eight times more energy-intensive than the Haber-Bosch process, while contributing to two of the three nitrogen atoms in each guanidine molecule. Thus, the overall energy cost for guanidine synthesis (from N_2) is dominated by the Cyanamide process, at about 94%. (8+8)/ (8+8+1) = 94%.

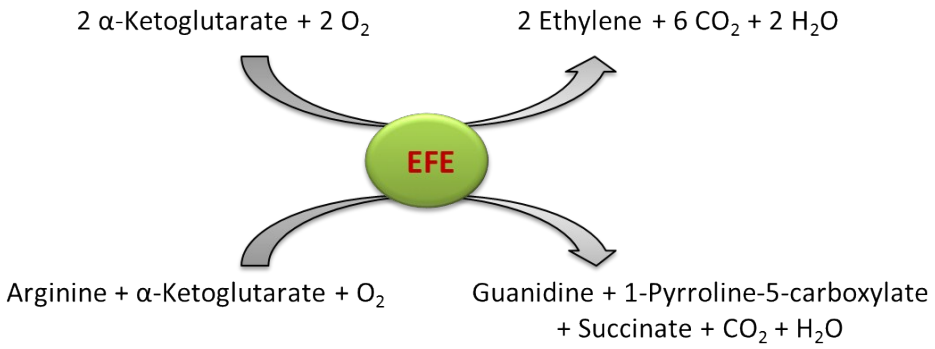


Figure S2 Reaction catalyzed by ethylene-forming enzyme (EFE).[3]

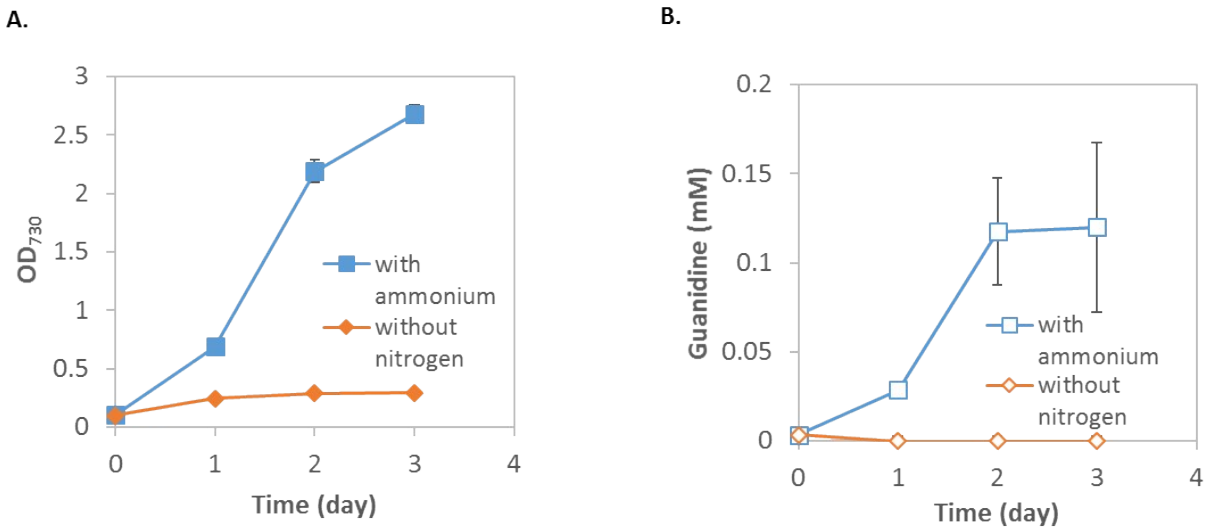


Figure S3 Guanidine production by the engineered *Synechocystis* strain PB646 (2x *efe*) using 5 mM NH_4Cl as the nitrogen source or in absence of nitrogen source. "w/ ammonium" stands for 5 mM NH_4Cl (instead of $NaNO_3$) in mBG11 medium as the nitrogen source. "no nitrogen" stands for absence of nitrogen source in the mBG11 medium, *i.e*, $NaNO_3$ removed from the defined mBG11 medium.

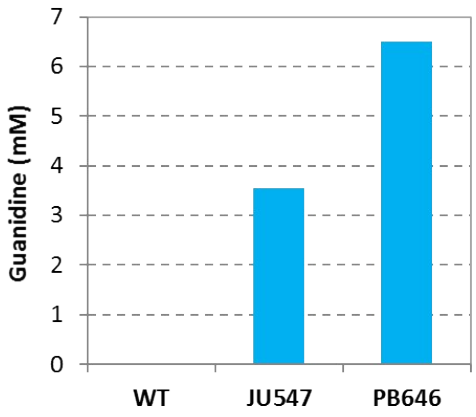
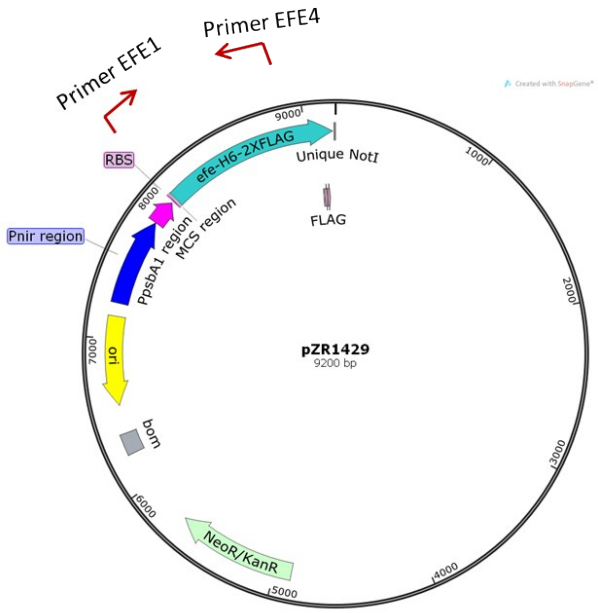


Figure S4 Intracellular levels of guanidine in *Synechocystis* strains.

A.

B.



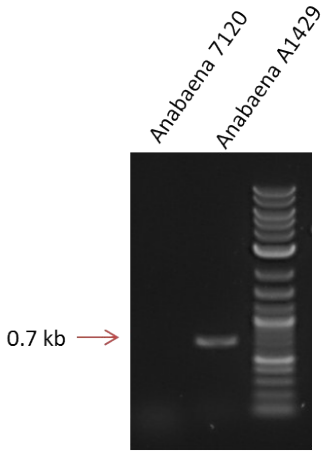


Figure S5 (A) Plasmid pZR1429 for EFE expression in *Anabaena*. (B) Genetic confirmation of *efe* in the *Anabaena* A1429 strain via colony PCR using primers EFE1 and EFE4.

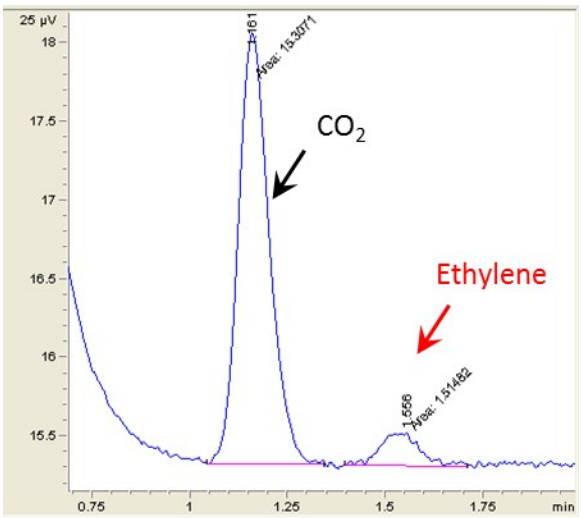


Figure S6 Production of ethylene from the engineered *Anabaena* strain A1429. 2 mL of culture was incubated overnight with 50 mM sterile bicarbonate in a 13 mL Hungate tube under a light of 15 µE m^{-2} s^{-1} at 30 °C. 500 µL headspace sample was analyzed on GC.

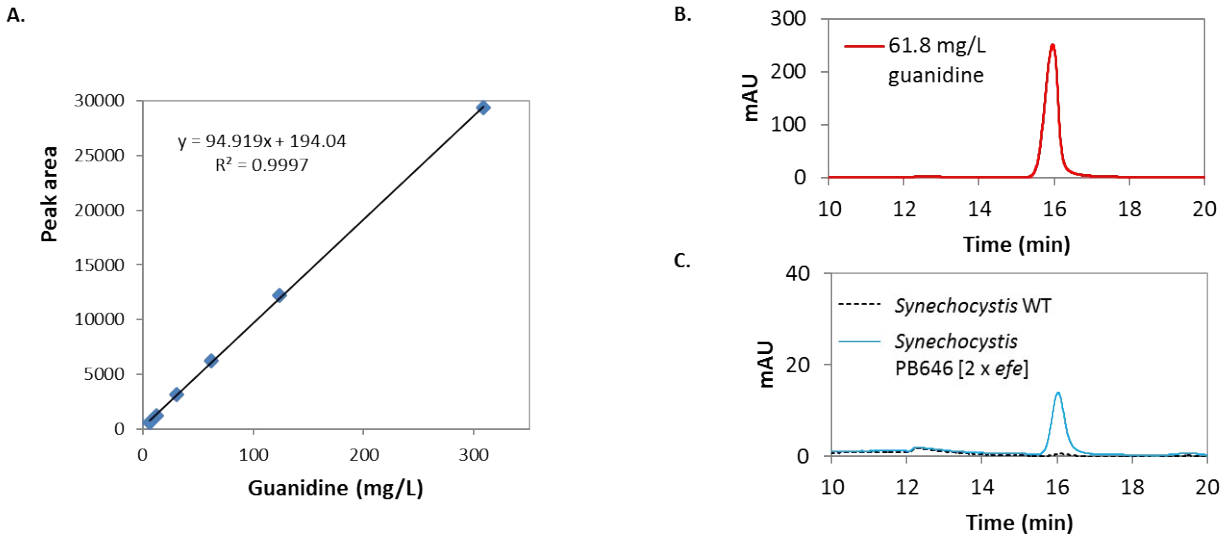


Figure S7 Analysis of guanidine using HPLC. (A) Guanidine standard curve. (B) HPLC signal for 61.8 mg L^{-1} guanidine. (C) An example of HPLC signals for the wild type (WT) and the *efe*-expressing (2x *efe*) *Synechocystis* strain PB646. Note that the retention time of guanidine may vary due to presence or absence of buffering compounds, such as *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) and $NaHCO_3$ in the samples. When a batch of biological samples were analyzed on HPLC, the guanidine standards were always included and were prepared using the same medium as in the culture.

Table S1 Enzymatic reactions consisting the guanidine biosynthesis (GUB) cycle.

Reactions		Enzymes/Genes in *Synechocystis*[4-6]
1	glutamate + N-acetyl-ornithine → N-acetyl-glutamate + ornithine	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase; ArgJ; *sll1883*
2	N-acetyl-glutamate + ATP → N-acetylglutamyl-phosphate + ADP	acetylglutamate kinase; ArgB; *slr1898*
3	N-acetylglutamyl-phosphate + H^+ + NADPH → N-acetyl-glutamate 5-semialdehyde + Pi + $NADP^+$	N-acetylglutamylphosphate reductase; ArgC; *sll0080*
4	N-acetyl-glutamate 5-semialdehyde + glutamate → N-acetyl-ornithine + α-ketoglutarate	acetylornithine aminotransferase; ArgD; *slr1022*
5	CO_2 + H_2O + glutamine + 2 ATP → carbamoyl-phosphate + glutamate + 2 ADP + 2 H^+ + Pi	carbamoyl phosphate synthase; CarAB; *sll1498*, *sll0370*
6	carbamoyl-phosphate + ornithine → citrulline + H^+ + Pi	ornithine carbamoyltransferase; ArgF; *sll0902*
7	citrulline + aspartate + ATP → arginino-succinate + AMP + H+ + PPi	arginosuccinate synthetase; ArgG; *slr0585*
8	arginino-succinate → fumarate + arginine	argininosuccinate lyase; ArgH; *slr1133*
9*	3 α-ketoglutarate + 3 O_2 + arginine → 2 ethylene + guanidine + 1-pyrroline-5-carboxylate + succinate + 7 CO_2 + 3 H_2O	ethylene-forming enzyme; EFE; *efe* [3]
10	1-pyrroline-5-carboxylate → glutamate-5-semialdehyde	spontaneous
11	glutamate-5-semialdehyde + NAD^+ + H_2O → glutamate + NADH + H^+	glutamate-5-semialdehyde dehydrogenase; PutA; *sll1561*
12	glutamate + NH_4^+ + ATP → glutamine + ADP + H^+ + Pi	glutamine synthetase; GlnA, GlnN; *slr1756*, *slr0288*
13	glutamine + α-Ketoglutarate + NADPH + H^+ → 2 glutamate + $NADP^+$	glutamate synthase; GltB, GltD; *sll1499*, *sll1502*, *sll1027*
14	fumarate + H_2O → malate	fumarase; FumC; *slr0018*
15	malate + NAD^+ → oxaloacetate + NADH + H^+	malate dehydrogenase; Mdh; *sll0891*
16	oxaloacetate + glutamate → aspartate + α-Ketoglutarate	aspartate transaminase; AspC; *sll0938*

* The reaction catalyzed by the EFE variant (*e.g.* A198V [7]) is: arginine + α-ketoglutarate + O_2 + → guanidine + 1-pyrroline-5-carboxylate + succinate + CO_2 + 3 H_2O

Table S2 Primers used in this study.

Primers	DNA sequence (5'-> 3')	Targets
PetE-F1	GGATTTAGGGGGATAAATACGGATCCAACACCACTGGGCCTACTG	*efe* expression cassette
T7-R2	CCTTTCGTTTTATTTGACGCGTCGCCAGATCCGGATATAGTTC	*efe* expression cassette
Kan9	GTCAACGCGTCCTAACTACGGCTACACTAGAAG	Kan^R
Kan2	ACGGTCGACCAGGTGGCACTTTTCGGGG	Kan^R
rT1KP	GTCAACGCGTCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGC CTTTCGTTTTATCTGCCTAACTACGGCTACACTAGAAG	*rrnB T1* and Kan^R
EFE1	ATGACCAATTTGCAAACTTTTGAATTAC	*efe*
EFE4	GTCCAGGGTTCGTCATG	*efe*
ZR45	cctcgtagaactagcaaag	P_{nir}
ZR791	tctcgagtaaggagagatctatATGACCAATTTGCAAACTTTTG	*efe*
ZR792	actagtcgacatgatgatgatgatgatgGCTACCAGTAGCGCGGGTGTCAC	*efe*

References

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2. Patil, B. S.; Hessel, V.; Seefeldt, L. C.; Dean, D. R.; Hoffman, B. M.; Cook, B. J.; Murray, L. J., Nitrogen fixation. In *Ullmann's Encyclopedia of Industrial Chemistry*, 2017.
3. Fukuda, H.; Ogawa, T.; Tazaki, M.; Nagahama, K.; Fujii, T.; Tanase, S.; Morino, Y., Two reactions are simultaneously catalyzed by a single enzyme: the arginine-dependent simultaneous formation of two products, ethylene and succinate, from 2-oxoglutarate by an enzyme from Pseudomonas syringae. *Biochem. Biophys. Res. Commun.* **1992,** *188* (2), 483-9.
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