

Supporting Information

Modulation of the intracellular O₂ concentration in *Escherichia coli* strains using Oxygen Consuming Devices (OCDs)

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Supporting Materials and Methods

Table S1. Oligonucleotide used in this work.

Primer name	Sequence 5' → 3'	Reference
VF2	TGCCACCTGACGTCTAAGAA	1
VR	ATTACCGCCTTTGAGTGAGC	
Bblac_F2*	ACCATC <u>GAATTC</u> GC GGCCGCTTCTAGAGGATGCAACGTCGTG	This work
Bblac_R2*	TCTTTACTAGTAGCGGCCGCTGCAGGGTTATACCGTAAACC	
Ec_cueO_F	ATCAACCTGCCGCTACCTGC	
Ec_cueO_R	TGTTGGCATGGTGGAAATCG	

*BioBrick prefix and suffix sequences are underlined and restriction sites highlighted in blue.

(1) Shetty, R. P., Endy, D., and Knight Jr., T. F. (2008) Engineering BioBrick vectors from BioBrick parts. *J Biol Eng* 2, 5.

Supporting Figures

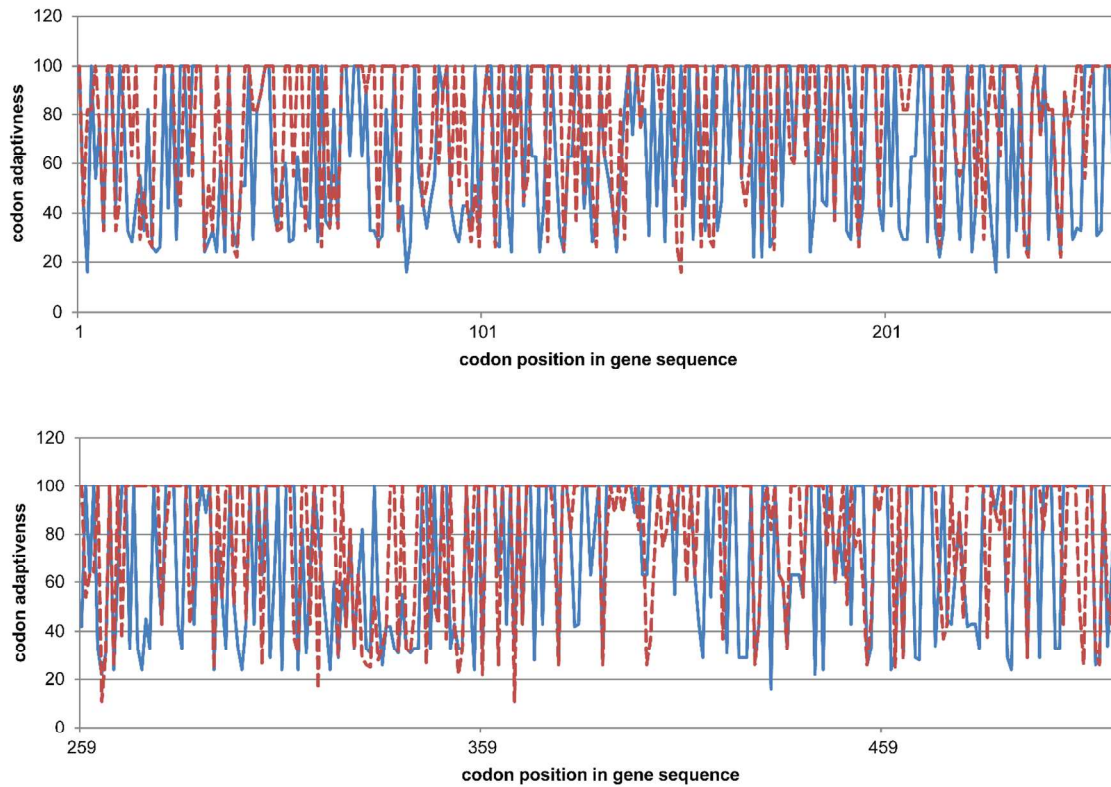


Figure S1. Comparison of the codon adaptiveness of the codon optimized (blue line) and the original (red dashed line) *cueO* ORFs. Codon adaptiveness along the codon position in gene sequence is plotted.

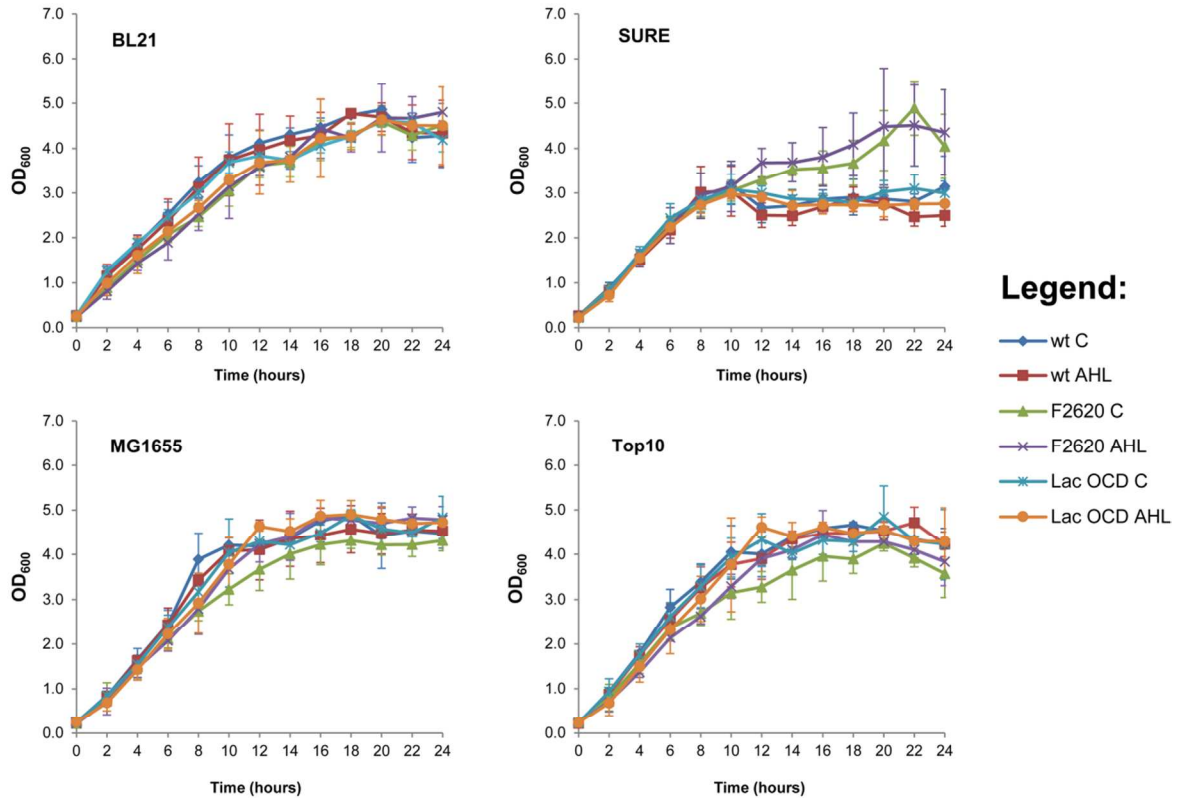
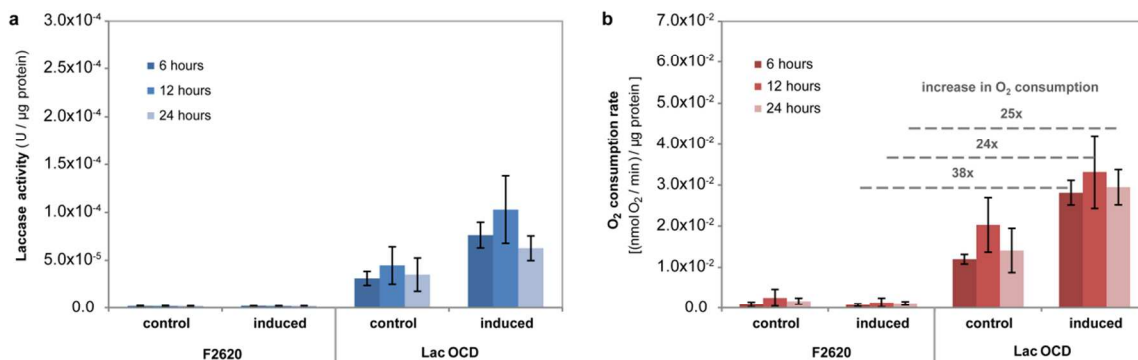


Figure S2. Effect of the Lac OCD activation on *E. coli* BL21, MG1655, SURE and Top10 growth. The wild-type (wt), the cells containing only the F2620 or F2620 plus the Lac OCD (Lac OCD) were grown in absence (C) or presence of the inducer (AHL). Growth was monitored measuring the OD₆₀₀ every 2 hours for a 24 hours period. Error bars represent the standard deviation of biological replicates ($n = 3$).

Exponential phase cells



Stationary phase cells

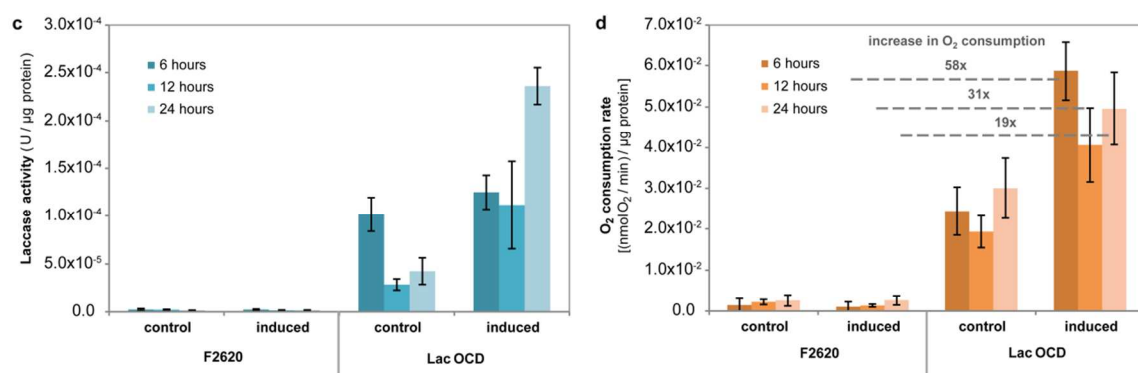


Figure S3. Characterization of the Lac OCD in *E. coli* DH5 α cells grown in M9 medium. Specific laccase activity (**a** and **c**) and O₂ consumption rate measurements (**b** and **d**) were performed using protein extracts obtained from cultures harboring the F2620 or the F2620 plus the Lac OCD (Lac OCD). Cultures were induced in exponential (top panel) or stationary phase (lower panel) and collected 6, 12 and 24 hours after induction. Cells were grown in the absence of inducer (control) or with 10 μM AHL (induced). The fold increase in O₂ consumption (induced Lac OCD vs. induced F2620) at 6, 12 or 24 hours after induction is shown in **b** and **d**. Results were normalized per μg of protein. Error bars represent the standard deviation of biological replicates ($n = 3$).

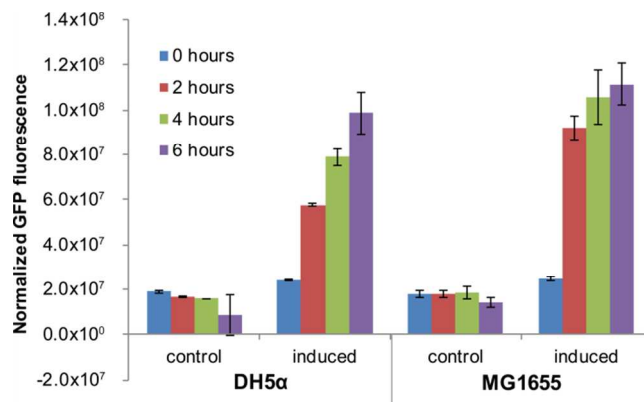


Figure S4. Normalized GFP fluorescence of *E. coli* DH5α and MG1655 cultures harboring the F2620 BioBrick + *gfp*. 96-well plates were setup using DH5α and MG1655 cultures ($OD_{600} = 0.1$) that were grown in the absence of inducer (control) or presence of 10 μM AHL (induced). Measurements were performed 0, 2, 4, and 6 hours after plate setup and the fluorescence was normalized to Abs_{620} . The results are representative of three biological replicates (with exception of MG1655 induced cultures, $n = 10$), with technical duplicates (measured in duplicate), error bars show \pm S.D.

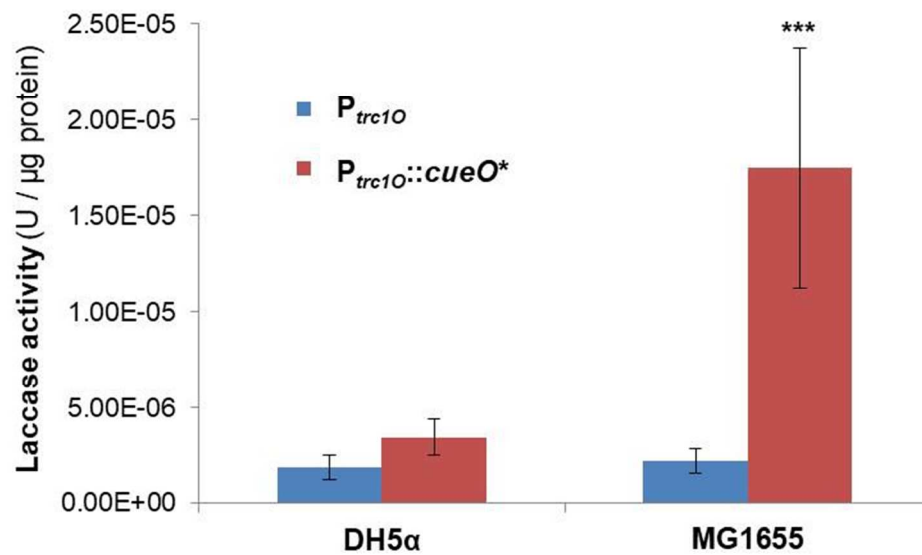


Figure S5. Characterization of the Lac OCD reassembled with the P_{trc10} promoter in *E. coli* DH5α and MG1655. Specific laccase activity was measured using protein extracts obtained from cells harboring the P_{trc10} or P_{trc10} plus the Lac OCD ($P_{trc10}::cueO^*$) and grown in M9 minimal medium supplemented with Cu^{2+} . Cultures were harvested 18 - 24 hours after inoculation. Results were normalized per µg of protein. Error bars represent the standard deviation of biological replicates ($n = 4$), *** represents p-value < 0.001.

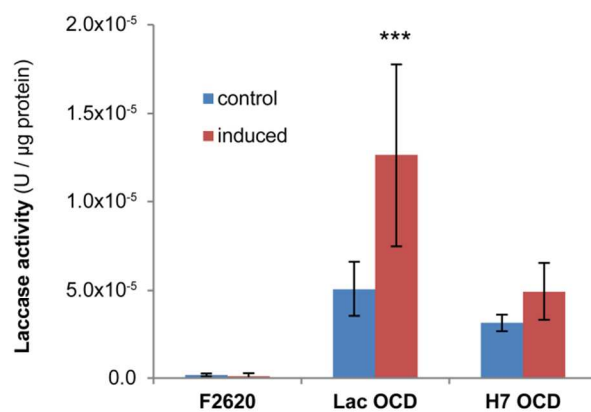


Figure S6. *In vitro* characterization of the Lac OCD in *E. coli* DH5 α cells using L-DOPA as substrate. Specific laccase activity was measured using protein extracts obtained from the cultures used in the *in vivo* characterization. Cells harboring the F2620, the F2620 plus the Lac OCD (Lac OCD) or the F2620 plus the Lac H7 variant (H7 OCD) were grown in M9 medium supplemented with Cu²⁺ in control and induced conditions (presence or absence of AHL, respectively). Results were normalized per μg of protein. Error bars represent the standard deviation of biological replicates ($n = 3$), *** represents p-value < 0.001 .

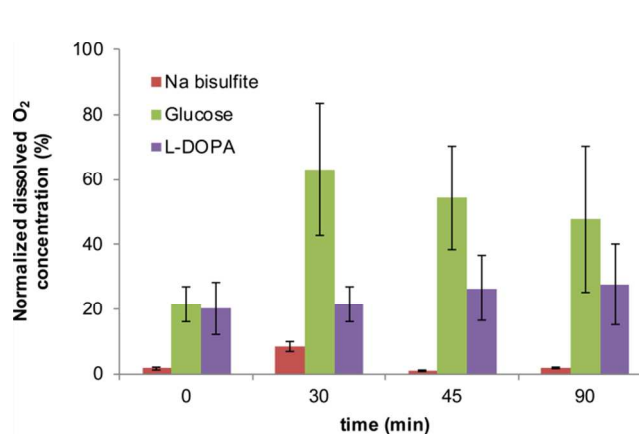


Figure S7. Dissolved O₂ measurements using *E. coli* Top10 suspensions incubated in sealed flasks. The cell suspensions were incubated with 30 mM L-DOPA (laccase substrate), 20 mM glucose (control for O₂ consumption) and 100 mM sodium bisulfite (Na bisulfite, control for O₂-deprived conditions) at 37 °C with shaking. One mL samples were removed at different time points (0, 30, 45 and 90 min) using sterile syringes and needles; and the dissolved O₂ concentration was measured using a Clark-type O₂ electrode. Results were normalized to the dissolved O₂ in saturated conditions (corresponding to 100% dissolved O₂). Error bars represent the standard deviation of biological replicates ($n = 2$).

Supporting Methods

GFP Fluorescence Analysis. For the evaluation of GFP expression, cultures of *E. coli* DH5 α and MG1655 harboring the F2620 BioBrick or the F2620::*gfp* were grown overnight (final OD₆₀₀ \approx 3) in 50 mL flasks at 37 °C with shaking (120 rpm). The cultures were diluted to a final OD₆₀₀ \approx 0.2 and 100 μ L aliquots were distributed in Nunc™ MicroWell™ 96-Well Optical-Bottom Plates (Thermo Fisher Scientific) containing 100 μ L of M9 medium or M9 with 20 μ M of AHL (to obtain a final concentration of 10 μ M AHL in 200 μ L). The 96-wells plates were incubated at 37 °C with shaking (120 rpm), and measurements were carried out in duplicate 0, 2, 4 and 6 hours after plate setup. GFP fluorescence and Abs₆₂₀ were detected using the Synergy 2 Multi-Mode Microplate Reader and the Gen5™ software (BioTek Instruments, Winooski, VT, USA). For fluorescence detection, an excitation filter of 485/20 nm and an emission filter of 528/20 nm were used (sensitivity set for 110). The experiments included 3 biological replicates, with the exception of MG1655 harboring the F2620::*gfp* for which 10 biological replicates were analyzed and technical duplicates were included for all samples. For data analysis, the background fluorescence and absorbance of the M9 medium was subtracted from the values obtained for the samples and, the fluorescence values were normalized by optical density.

Dissolved O₂ Concentration Measurements Using Sealed Cultures. Cultures of *E. coli* Top10 harboring the OCD (grown overnight) were washed and resuspended in 50 mM phosphate buffer, pH 7.0 (KPi) to a final OD₆₀₀ \approx 1.0. This cell suspension was distributed by different 4 Erlenmeyers (100 mL): the oxygen-saturated medium control (containing only cells), the oxygen-deprived medium control (cells + 100 mM sodium bisulfite) and the other two contained 20 mM L-DOPA (laccase substrate) or 20 mM glucose (used as O₂ consumption control). The Erlenmeyer's were sealed using Suba Seal rubber stoppers (Sigma), and were incubated at 37 °C with shaking. At different time points (0, 30, 45 and 90 min), 1 mL samples were removed through the stopper septa using a sterile syringes and needles and, the dissolved O₂ concentration was measured using a

Clark-type O₂-electrode (Hansatech Instruments). Results were normalized to the dissolved O₂ in saturated conditions (corresponding to 100% dissolved O₂).