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Nitrous oxide (N_2O) synthesis by the freshwater cyanobacterium *Microcystis aeruginosa*

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S1. Specific N_2O production rates

Table S1.1. N_2O production rates (nmol $N_2O \cdot g\ DW^{-1} \cdot h^{-1}$) recorded from the linear regressions performed for each *M. aeruginosa* biomass concentrations (0.1, 0.2 and 0.4 g-DW·L^{-1})

Initial biomass (g-DW·L^{-1})	**N_2O (nmol $N_2O \cdot g\text{-}DW^{-1} \cdot h^{-1}$)**	**R^2**
0.1	128	0.99
0.2	123	0.97
0.4	124	0.93

S2. Enzymatic kinetic of N_2O synthesis in *Microcystis aeruginosa*

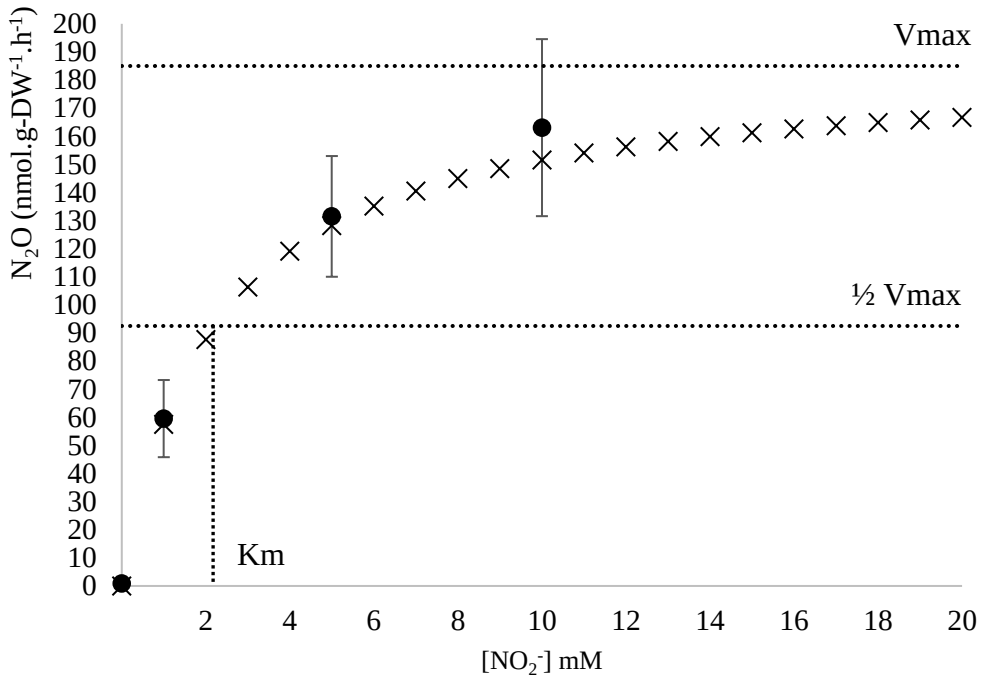

Figure S2.1. Impact of initial NO_2^- concentration (0, 1, 5 and 10 mM; n ≥ 7, n = 18, n = 16, n = 23, respectively) on the N_2O production in *M. aeruginosa* cultures incubated 24 hours in light (●). The observed kinetic followed a Michaelis-Menten kinetic (×): Vmax and Km were graphically estimated at 185 nmol·g-DW^{-1}·h^{-1} and 2.22 mM, respectively and used to simulate the Michaelis-Menten kinetic based on the equation described by (Johnson and Goody, 2011).

S3. Confirmation of the purity of *M. aeruginosa* cultures.

To confirm the purity of the *Microcystis aeruginosa* cultures used during the experiment, DNA was extracted from cultures that had undergone different treatments:

1. a culture grown in an E-flask, subsequently used to perform bioassays.
2. a culture grown in Duran bottle (as described in the manuscript), subsequently used to perform bioassays.

Bioassays were performed as described in the manuscript (i.e. centrifugation, re-suspension in N-free media and NO_2^- supplementation) and DNA was extracted from the mother culture and at the beginning (time = 0h) and the end of bioassays (48 h). For each condition, a 25 mL culture aliquot was withdrawn and centrifuged at room temperature at 4400 rpm for 3 min. The supernatant was discarded and the cultures were frozen at -80^{o}C. The DNEasy Blood and Tissue Kit (Qiagen, Germantown, MD) was used for DNA extraction. About 90 µl of thawed pellet was added to 180 µl of ATL buffer and 20 µl of proteinase K, from the kit. The mixture was vortexed and incubated at 55^{o}C overnight. Following the kit protocol, extracted DNA was finally eluted in 100 µl of purified water. DNA concentration was determined using a Qubit 2 fluorometer (ThermoFisher Scientific) and varied between 12.2 and 28.9 ng/µl.

An aliquot of each template DNA was diluted to a concentration of 2 ng/µl to make a working stock for Polymerase Chain Reaction (PCR). Primers for the bacterial 16S rRNA gene (27F: AGAGTTTGATCMTGGCTCAG and 1492R: CGGTTACCTTGTTACGACTT; (Lane, 1991) were used to amplify a fragment of approximately 1.4 kb from the cyanobacterial DNA in a PCR mix that contained 2 µl of 2 µM forward and reverse primers (final concentration 0.2 µM), 10 µl of Platinum^{TM} II Hot Start PCR Master Mix (ThermoFisher Scientific), 4 ng of template DNA and water to a final volume of 20 µl. Two negative reactions were included. The PCR cycling program was as follows: 95^{o}C for 2 min; 35 cycles of (95^{o}C for 15 sec; 55^{o}C

for 15 sec; 68ºC for 30 sec) followed by a "hold" at 10ºC. The resulting fragments were visualized by electrophoresis (1% Agarose gel in TAE with 1X SyBr Safe -Invitrogen) at 100V for 40 min and photographed using a UVIDOC gel-doc (UVITEC, Cambridge). The gel showed 8 single fragments of the expected size and no products in the negative controls (**Figure SI.1**).

Figure S1.1. Photograph of the electrophoresis showing the PCR product of each sample. 1, 2: Culture from a Duran bottle used for bioassays (0 h); 3, 4: Culture from an E-flask used for bioassays (0 h); 5, 6: Cultures from a Duran bottle used for bioassays (48 h after the addition of 10 mM NO_2^-); 7,8: Cultures from an E-flask used for bioassays (48 h after the addition of 10 mM NO_2^-), 9 and 10: negative controls (PCR mixture without DNA).

The PCR reactions were cleaned for sequencing by the addition of 0.5 µl (0.5U) of Shrimp Alkaline Phosphatase (rSAP, New England BioLabs) and 0.25 µl (5U) of Exonuclease I (EXO1, New England BioLabs) and incubating at 37ºC for 15 mins followed by a heat kill at 85ºC for 15 mins.

Fragments were sequenced in both directions using the forward and reverse primers that were used for PCR. The sequencing was carried out on an ABI 3730 capillary machine (Perkin Elmer) in the Massey Genome Centre, Palmerston North. Electrophoretograms were

assembled and edited using Geneious 9.1.2 (https://www.geneious.com). The sixteen resulting electrophoretograms did not show any contaminating dye peaks that would make sequence determination difficult or impossible. The forward and reverse sequences were assembled and aligned and were found to be identical with no unresolved ambiguities. The consensus sequence was exported (see below) and matched sequences of *Microcystis aeruginosa* in a GenBank megablast search (https://blast.ncbi.nlm.nih.gov).

>consensus sequence
TGCAAGTCGAACGGGAATCTTCGGATTCTAGTGGCGGACGGGTGAGTAACGCGT
AAGAATCTAACTTCAGGACGGGGACAACAGTTGGAAACGACTGCTAATACCCGA
TGTGCCGCAAGGTGAAACCTAATTGGCCTGAAGAAGAGCTTGCGTCTGATTAGCT
AGTTGGTGGGGTAAGAGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGA
TGAGCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAG
TGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGSGTGAGGGA
GGAAGGTCTTTGGATTGTAAACCTCTTTTCTCAAGGAAGAAGTTCTGACGGTACT
TGAGGAATCAGCCTCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGGGGAG
GCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGTCCGCAGGTGGTCAGCCAAG
TCTGCCGTCAAATCAGGTTGCTTAACGACCTAAAGGCGGTGGAAACTGGCAGAC
TAGAGAGCAGTAGGGGTAGCAGGAATTCCCAGTGTAGCGGTGAAATGCGTAGAG
ATTGGGAAGAACATCGGTGGCGAAAGCGTGCTACTGGGCTGTATCTGACACTCA
GGGACGAAAGCTAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTCCTAGCCGT
AAACGATGGATACTAGGCGTGGCTTGTATCGACCCGAGCCGTGCCGAAGCTAAC
GCGTTAAGTATCCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAWCGCGA
AGAACCTTACCAAGACTTGACATGTCGCGAACCCTGGTGAAAGCTGGGGGTGCC
TTCGGGAGCGCGAACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA
TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTTAGTTGCCAGCATTAA
GTTGGGGACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA
CGTCAAGTCAGCATGCCCCTTACGTCTTGGGCGACACACGTACTACAATGGTCGG
GACAAAGGGCAGCGAACTCGCGAGAGCCAGCGAATCCCAGCAAACCCGGCCTC
AGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGGAGGAATCGCTAGTAATC
GCCGGTCAGCATACGGCGGTGAATTCGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGGAAGCTGGTCACGCCCGAAGTCATTACCTCAACCGCAAGGAGGGGG
ATGCCTAAGC

Reference

Lane, D. J. (1991). 16S/23S rRNA sequencing, p. 115-175. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. *John Wiley & Sons*, New York.