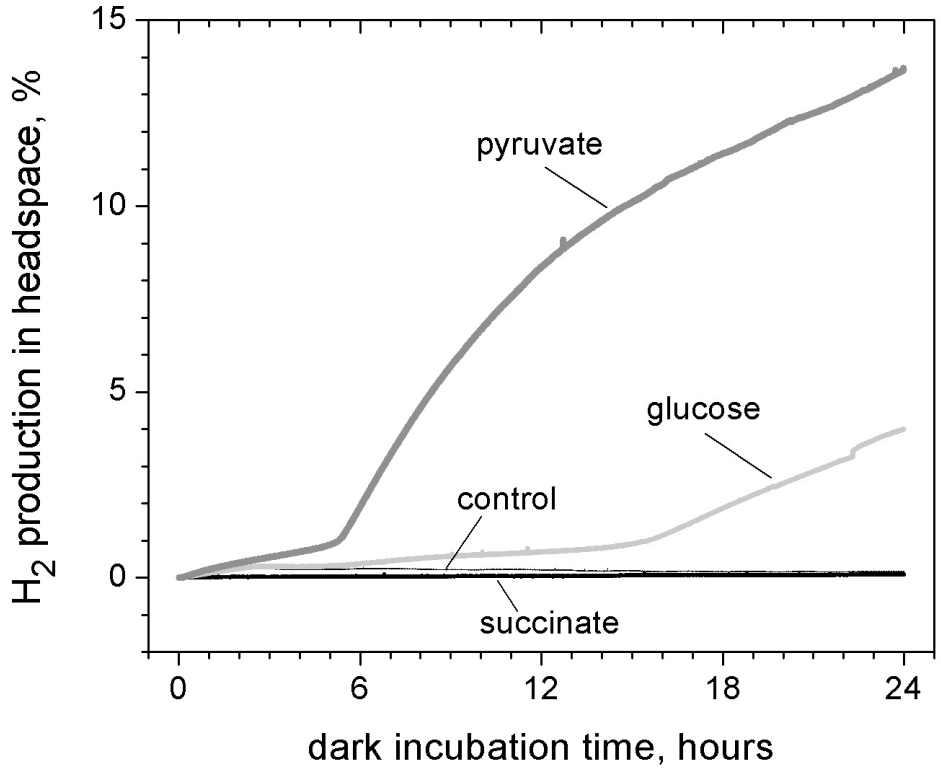
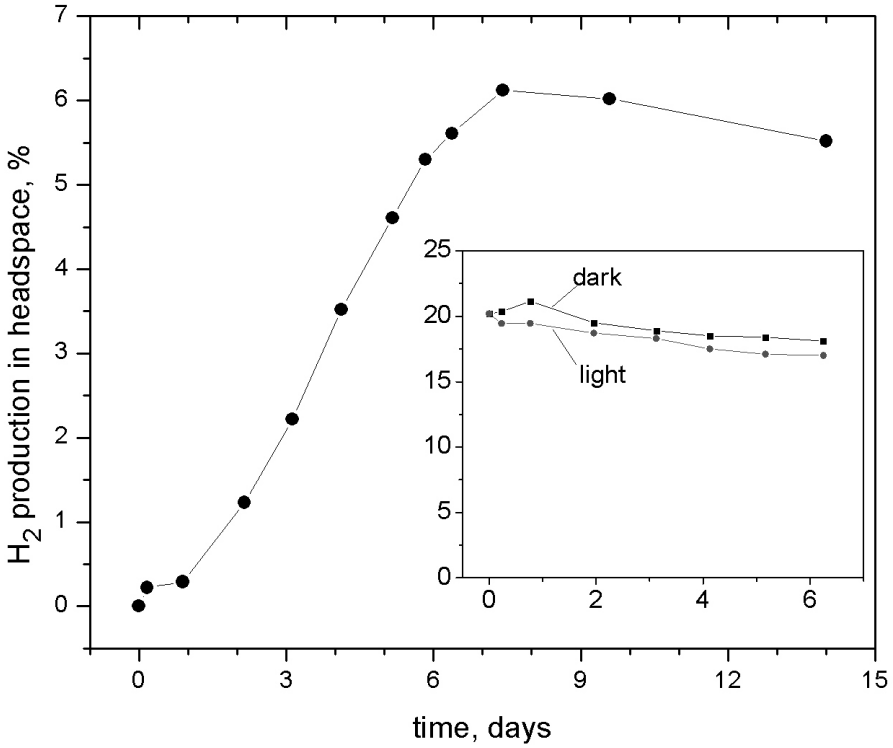
Optimizing Metabolic Capacity and Flux through Environmental Cues to Maximize Hydrogen Production by Cyanobacterium *Arthrospira* (*Spirulina*) *maxima*

Gennady Ananyev, Damian Carrieri and G. Charles Dismukes

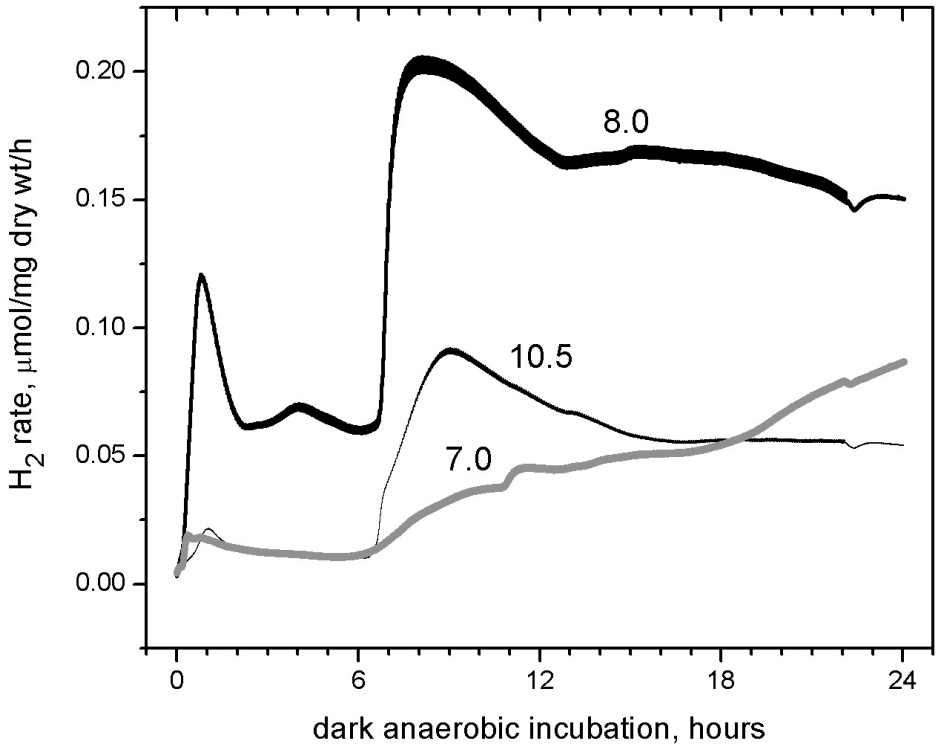
Department of Chemistry, Princeton University, and Princeton Environmental Institute
Princeton, NJ 08544 Phone: (609) 258-3896, Fax: (609) 258-1980,
E-mail: dismukes@princeton.edu



S-1. Dark H_2 production by *Arthrospira maxima* in the presence of exogenously added substrates (at time 0): pyruvate (10mM), glucose (20mM), and succinate (10mM) or no additions (control). H_2 was measured in a bioreactor device consisting of a round-bottom flask (100 mL) with 50mL of cells at moderate density (~1.5 g dry weight/L) interfaced with a Clark-type electrode for continuous monitoring of H_2 in the headspace, previously calibrated with gaseous standards.



S-2. Dark H_2 production by *A. maxima* cells grown photoautotrophically for 1 month (12 days stage 1, 18 days stage 2) in batch culture as described in Materials and Methods. The sample volume is 25 mL, the headspace gas volume is 33 mL. Zarrouk's medium was additionally supplemented with 1 μM Ni^{2+} as previously described (12). Inset shows the results of illuminating (white light, PFD = ~70 μE m^{-2} s^{-1}) and darkness on the H_2 concentration in the headspace after first purging with 20 % H_2 + 80 % N_2 at the beginning of anaerobisis.



S-3. Dark anaerobic H_2 production by *A. maxima* cells grown photoautotrophically at different pH values in Zarrouk's medium, pH adjusted by addition of HCl (to lower pH to 7.0 or 8.0), and measured in the same medium.