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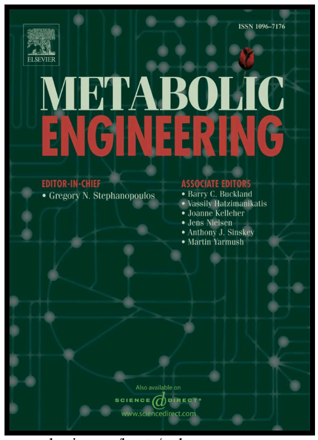

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Improved [FeFe] hydrogenase O_2 tolerance suggests feasibility for photosynthetic H_2 production

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ABSTRACT
Photosynthetic H_2 production has been a compelling but elusive objective. Here we describe how coordinated bioreactor, metabolic pathway, and protein engineering now suggest feasibility for the sustainable, solar-powered production of a storable fuel to complement our expanding photovoltaic and wind based capacities. The need to contain and harvest the gaseous products provides decisive solar bioreactor design advantages by limiting O_2 exposure to prolific, but O_2-sensitive H_2 producing enzymes—[FeFe] hydrogenases. CO_2 supply and cell growth can also be limited so that most of the photosynthetic reduction capacity is directed toward H_2 production. Yet, natural [FeFe] hydrogenases are still too O_2 sensitive for technology implementation. We report the discovery of new variants and a new O_2 tolerance mechanism that significantly reduce the sensitivity to O_2 exposure without lowering H_2 production rates or losing electrons to O_2 reduction. Testing the improved hydrogenases with a biologically derived, light-dependent electron source provides evidence that this game changing technology has the potential for sustainable large-scale fuel production.

Keywords: [FeFe] hydrogenase; O_2 tolerance; Hydrogen; Renewable energy; Biofuel

1. Introduction

Producing H_2 from sunlight and water as a sustainable fuel source has been a long standing but difficult objective. Successful photosynthetic H_2 production requires coordinated advances in both bioreactor and organism design as well as protein engineering. Because the product accumulates as a gas, the culture must be fully contained (Fig. 1A). While this requirement may increase initial capital costs, it also confers crucial metabolic advantages. Because air is excluded, the only source of O_2 is from water splitting. The available thermodynamic driving force (photon free energy) will limit achievable H_2 partial pressures (P_{H2}, approximately 0.05 atm), and reaction stoichiometry will limit P_{O2} to half of P_{H2}. This limitation will avoid the significant loss of reducing equivalents to Mehler reactions which occur during normal photosynthesis—especially under intense solar illumination (Makino et al., 2002; Roberty et al., 2014). In addition, limiting both P_{CO2} and P_{O2} will dramatically lower reducing equivalent consumption by RuBisCO (Quintana et al., 2011) thereby directing most of the electron flux toward H_2 production by a prolific enzyme, an [FeFe] hydrogenase. Avoiding the need for carbon fixation, which requires the conversion of very low partial pressure CO_2 into reduced biochemical, dramatically increases the