Discovery of [FeFe] hydrogenase variants with enhanced O_2 tolerance

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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.