Laccase-Catalyzed Bioelectrochemical Oxidation of Water Assisted with Visible Light.

Cristina Tapia^a, Sergey Shleev^b, Jose Carlos Conesa^a, Antonio L. De Lacey^{a*}, Marcos Pita^{a*}.

^a Instituto de Catálisis y Petroleoquímica, CSIC. C/ Marie Curie, 2. L10 28049 Madrid, Spain.

^b Biomedical Sciences, Faculty of Health and Society, Malmö University, SE-0205 06 Malmö, Sweden.

KEYWORDS : photocatalysis, biocatalysis, laccase, water splitting, oxygen, visible light.

DIGITAL CSIC version. Originally published at ACS Catalysis 2017, 7, 4881–4889: DOI 10.1021/acscatal.7b01556

ABSTRACT: Here we present the modification of fluorinated tin oxide electrodes with In₂S₃, a n-type semiconductor chalcogenide that absorbs visible light ($\lambda \leq 600\text{nm}$), and its further use as an active scaffold for laccase-catalyzed oxidation of water. Illumination of FTO-In₂S₃-laccase electrode yields O₂ production at much lower applied potential than the previous example using the same laccase, where only electric energy was applied. The present system allows a diversification of the energy applied to accomplish the water splitting, taking a portion of it from the Sun. This work is the first example where an enzyme other than PSII has been used combined with visible light to biocatalyze the O₂ evolution.

Introduction

Solar and wind energies, which play an increasingly important role for a sustainable development, suffer of an intermittency problem which demands some sort of storage. One way to solve it is to capture CO₂ or decompose water through processes such as

or

This allows storing energy as fuel. In the case of solar energy these processes are usually named solar fuel generation or artificial photosynthesis, and were revealed as possible for the first time by work by Fujishima and Honda in the early seventies^{1,2} showing that water could be split in H₂ and O₂ using a TiO₂ photoelectrode. Because of the importance and potential of the concept many reviews³⁻⁷ have been devoted to this subject. Photoelectrochemistry has advantages against pure photocatalysis, since it facilitates the fuel and O₂ production in separate compartments (avoiding thus their recombination); and also against photovoltaics+electrolysis as it may diminish the energy losses due to the lower number of interfaces that charge carriers must go through. Furthermore, electrochemistry and photoelectrochemistry of semiconductors had already been deeply studied,⁸⁻¹¹ building a sound scientific basis to understand and develop that technology. The study of photocatalysis by oxides had begun much earlier¹² and provides additional knowledge on the surface chemistry involved.

The production of solar fuels needs a compound able to capture solar light and separate the photogenerated positive (holes) and negative (electron) charges so that chemistry can be made from them. Besides, the resulting redox levels must have potential enough to produce the desired chemistry. For water splitting the potential difference must be higher than

1.23 V (E_0 for O₂ evolution at pH=0), implying that those energy levels should be separated by a significant extra energy to overcome the energy losses; in addition, the excited electrons should appear at energy higher than the H₂ evolution redox potential, and the holes at energies lower than the O₂ evolution redox potential. Many light absorbers i.e. molecular entities or polymers have been proposed and reviewed^{5,13-15}. Here we focus on semiconducting light absorbers with bandgap energy $E_g \geq 1.7$ eV. TiO₂ (rutile or anatase) is the best-known photocatalyst and photoelectrode: is stable, affordable and highly photoactive; however its large bandgap ($E_g=3.0\text{-}3.2$ eV) limits TiO₂ to UV light, disregarding $\geq 95\%$ of the solar spectrum. Huge efforts have been driven toward other materials able to use efficiently visible light. These include TiO₂ doped with cations (Cr, V, Fe) or anions (N or S); oxides like BiVO₄, Cu₂O, WO₃ or Fe₂O₃; nitrides or oxinitrides like Ta₃N₅, TaON, the perovskites LaTiO₂N and LaNbON₂ or two-dimensional g-C₃N₄; or sulphides like CuGaS₂, CdS, In₂S₃, ZnIn₂S₄, SnS₂ or MoS₂ although the mentioned sulphides and some of the nitrides are prone to photooxidation during solar fuel generation, needing coverage with protecting oxide layers and/or co-catalysts able to extract holes efficiently. Photon harvesting semiconductors have been frequently reviewed, and we refer the reader to those works.^{3,4,6,16-18}

Both reactions (1) and (2) release O₂ molecules, thus extract electrons from water to form O–O bonds. This oxygen electroevolution reaction (OER) is kinetically much more demanding than the generation of H₂ itself (even though the latter is the interesting product), due to the need of coordinated transfer of 4 electrons and 4 protons as well as oxygen atoms transport (heavier than H), which in addition bear higher electrostatic charges; thus the electrochemical overpotentials needed are relatively large. Efficient co-catalysts may facilitate such electrochemical process. Oxides like RuO₂ and IrO₂

are rather efficient but scarce and expensive elements co-catalysts in acid medium,¹⁹⁻²³ Under alkaline conditions some common metal oxides are an alternative.²⁴ Among them oxidized nickel, known to exist under working conditions as a relatively ill-crystallized oxyhydroxide containing Ni^{3+} , is recognized as a rather good OER catalyst, in particular when including some iron as detected long ago by Corrigan²⁵ and thoroughly verified recently;²⁶ the best OER catalyst to date might be the amorphous Fe-Co-W oxide reported by Zhang et al.,²⁷ for which the overpotential required to reach a current density of 10 mA/cm^2 (the parameter normally used to assess OER efficiency) is as low as 191 meV. Other materials reported are the cobalt phosphates proposed for the “artificial leaf”²⁸ or cobalt poly-oxometalates;²⁹ also, manganese and copper oxides or coordination complexes have been proposed³⁰⁻³³ inspired on the active centres of the natural enzymes known to catalyze efficiently the complete transformation between O_2 molecules and water: photosystem II, which facilitates OER in natural photosynthesis, and laccases, which reduce O_2 all the way to water without stopping the reaction at the H_2O_2 stage. In these cases the co-catalysts are arranged as nanoparticles, clusters or molecular complexes deposited on the surface of the light absorbing semiconductor, and must have electronic levels lower than the O_2 redox potential but higher than the valence band of the semiconductor so that holes photogenerated at the latter may be transferred efficiently to water or OH^- groups at the co-catalyst-liquid interface.

As there are enzymes able to mediate the electrochemical conversion between water and O_2 , such enzymes may also work as co-catalysts. Indeed several works have tried to immobilize photosystem II (PSII) on electrodes. PSII is a multi-subunit enzyme that performs solar light-driven H_2O oxidation in photosynthetic organisms. This protein complex contains, besides the photosensitive centres that absorb visible light and the cofactors in which charge formation and separation takes place, a Mn_4CaO_5 cluster where the OER is catalyzed.³⁴ Therefore, as reported by several research groups, when attached to an electrode the PSII can replace both photon absorbing substance and electrocatalyst to achieve the photoelectrochemical H_2O oxidation.³⁵⁻³⁷ However, in these cases the PSII was immobilized on the electrode either within a conductive polymer or in presence of a redox mediator in the electrolyte in order to transfer the electrons from the catalytic site of PSII to the electrode surface.³⁵⁻³⁷ The reason is that the large size, high structural complexity, and fragile stability of PSII, when isolated from its natural environment in the cellular membrane, do not favour fast direct electron transfer, as has been done with simpler metalloenzymes.^{38,39} Kato et al. have reported direct electron transfer of PSII by a covalent oriented immobilization of the enzyme on an indium tin oxide electrode (ITO) modified with a self-assembled monolayer,⁴⁰ but the photocurrent densities measured were two orders of magnitude smaller than those obtained by the same group, when the enzyme was wired to a ITO electrode by redox polymers.³⁷

In a previous work we showed that the above mentioned copper-containing laccase enzymes, the natural activity of which is the 4-electron reduction of O_2 in moderately acidic aqueous solutions, could catalyze the reverse reaction when forced under certain conditions.⁴¹ Laccases contain 4 Cu atoms distributed in two different sites of the enzyme: (i) the T1 site, which contains one Cu ion coordinated to 3 or 4 amino acid residues, is near the protein surface and in the O_2 reduction process receives the electrons from the donor compound dur-

ing the catalytic reaction; (ii) the T2/T3 site, which is formed by a cluster of 3 Cu ions and is the catalytic centre for the 4-electron reduction of O_2 to H_2O . An intramolecular electron pathway connects the T1 and T2/T3 sites and there are also channels for O_2 and H_2O transport between the protein surface and the T2/T3 cluster.⁴² Laccases are classified according to the redox potential of their T1 site.⁴³ The laccases with a high redox potential T1 site, which are of fungal origin, are very interesting for electrocatalytic applications because they are able to reduce oxygen at potentials above or near 0.7 V vs. SHE by direct electron transfer, produce high catalytic current densities and have high operational stability.⁴⁴⁻⁴⁹ The catalytic activity of laccases is irreversible under the normal conditions of measurement, which are a pH value around 4 and either an electron donor compound or an electrode biased at a redox potential lower than that of its T1 site (0.7-0.85 V for the high redox potential laccases).^{42,50} However, we have shown that a native and a mutant fungal laccases covalently attached to chemically modified graphite electrodes were capable of oxidizing H_2O to O_2 at neutral pH, when a high overpotential of +1.2 V vs. SHE was imposed at the electrode.⁴¹

In the present work we show that the overpotential for water oxidation catalyzed by *Trametes hirsuta* laccase can be considerably decreased in a photoelectrochemical device comprising a hybrid inorganic/biological photocatalyst formed by laccase covalently bound to In_2S_3 (Scheme 1). In_2S_3 is a n-type semiconductor with a band gap $E_g=2.0 \text{ eV}$, well within the energy range of visible light radiation, and its valence band has an energy level lower than that required for oxygen evolution at neutral pH.^{51,52} A photochemical study has revealed a carrier mobility value for In_2S_3 that is in the same range as compounds frequently used in photoelectrodes, such as the metal oxides semiconductors Cu_2O , WO_3 and BiVO_4 .^{53,54} In_2S_3 has been tested as photoanode in aqueous media, but only rarely without a sacrificial agent⁵⁵⁻⁵⁷, so that O_2 photogeneration may be feasible. However such works disregarded the photocorrosion or the generation of byproducts like H_2O_2 , which have been evidenced^{58,59}. Here we present a photoelectrochemical study of covalently-bound laccase molecules to In_2S_3 particles deposited on transparent fluorinated tin oxide (FTO) electrodes for O_2 evolution, detected with an appropriate sensor, under visible light illumination.

Scheme 1. Energy transfer diagram for the photo-assisted water oxidation bioelectrocatalytic process

This is, to our knowledge, the first study on the photoanodic behavior of In_2S_3 with an attached OER co-catalyst of any kind (inorganic or enzymatic), as well as the first work using a laccase as OER co-catalyst in a photoelectrochemical experiment.

Experimental

Reagents and materials. All the reagents were used as received without further purification. Di-sodium hydrogen phosphate 12-hydrate and sodium di-hydrate phosphate 2-hydrate 99.0-102.0% were purchased from Panreac. InCl_3 , Thiourea, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt 98% (ABTS), MES hydrate 99.5%, 4-nitrophenyldiazonium perchlorate, tetrabutylammonium tetrafluoroborate 99.0%, sodium meta-periodate 99.8%, n-(3-dimethylaminopropyl)-n'-ethylcarbodiimide hydrochloride (EDC), Type I horseradish peroxidase $147 \text{ U} \cdot \text{mg}^{-1}$ (HRP), and fluorine-doped tin oxide coated glass (FTO) were purchased from Sigma Aldrich. N-hydroxysuccinimide 97.0% (NHS) was purchased from Fluka. Ethanol absolute 99.5%, acetonitrile and acetone 99.5% were purchased from Scharlau. Aqueous solutions were prepared using MilliQ deionized water ($18.2 \text{ M}\Omega \times \text{cm}$). O_2 (g) and Ar(g) were purchased from Air Liquide. Grey dielectric paste was supplied by Gwent Group.

Trametes hirsuta laccase (ThLc), strain 56, was purified at Moscow State University of Engineering Ecology as previously reported⁶⁰. HPLC and SDS-PAGE showed a homogeneous purification of the enzyme. The enzyme concentration was determined by the Bradford method⁶¹ using a Bio-Rad Laboratories kit with bovine serum albumin for calibration.

Synthesis of In_2S_3 . Polycrystalline $\beta\text{-In}_2\text{S}_3$ was synthesized and characterized as reported previously⁶² following a known hydrothermal procedure⁶³.

Preparation of FTO- In_2S_3 electrodes. 2 x 1 cm FTO electrodes were cleaned by immersion into water and 15 min ultrasound treatment. Afterwards the electrodes were washed with EtOH and immersed into acetone, applying again 15 min ultrasounds. The electrodes were further washed with EtOH, immersed into an EtOH solution and applied ultrasounds during 15 min. The electrodes were let to dry. A copper wire was fixed to the FTO surface with conductive adhesive tape (Aris-

ton), and finally the electrodes were masked with dielectric paste leaving exposed a 5 mm diameter circular FTO surface.

A $4 \text{ mg} \cdot \text{mL}^{-1}$ suspension of In_2S_3 in ethanol was prepared and subjected to ultrasounds during 20 min. FTO electrodes were modified by dropping 7 μL of the In_2S_3 suspension and let to dry for 5 min. The dropping-drying process was performed a total of 5 times and finally let to dry overnight (Fig. 1).

Figure 1. Photograph of (a) FTO- In_2S_3 and (b) bare FTO electrodes.

X-Ray Diffraction (XRD) of FTO- In_2S_3 electrodes was performed with a Philips X'Pert Pro PANalytical diffractometer (Cu-K α , $\lambda = 0.1541874 \text{ nm}$). The samples were scanned from 4 to 90 degrees and the accumulation time was 20 s. No pretreatment of the samples was needed.

Scanning electron microscopy (SEM) images of FTO- In_2S_3 electrodes were obtained with a TM-1000 Tabletop Hitachi.

***Trametes hirsuta* laccase covalent immobilization on FTO- In_2S_3 electrodes.** FTO- In_2S_3 electrodes were immersed in a 3-electrode electrochemical cell filled with 5 mL of CH_3CN containing 2 mM of 4-nitrophenyldiazonium perchlorate and 100 mM Bu_4NBF_4 as electrolyte. Electrochemical grafting of nitrophenyl groups to the FTO- In_2S_3 electrodes was performed by recording two cyclic voltammograms (CVs) from 0.8 to 0.1 V vs. SHE at 100 mV s^{-1} scan rate. The modified electrodes were rinsed thoroughly with EtOH and taken into an electrochemical cell containing a 9:1 mixture of $\text{H}_2\text{O}:\text{EtOH}$. Two CVs were recorded from 0.2 to -1.2 V vs. SHE at $100 \text{ mV} \cdot \text{s}^{-1}$ scan rate to reduce the pendant nitro phenyl groups to amino phenyl ones.⁶⁴

3 μL pH 6.0 100 mM phosphate buffer containing 5.8 mg mL^{-1} ThLc were added to 33 μL of 47 mM NaIO_4 and let to incubate at 0°C during 30 min. Then, 54 μL of 100 mM phosphate buffer pH 7.1 were added to the solution. 15 μL of the resulting solution were deposited on the surface of an amino-aryl-modified FTO- In_2S_3 electrode at room temperature and let to incubate for 45 min. Afterwards, 13.5 μL of 14 mM EDC and 11.2 μL of 21 mM NHS were added to the electrode and let to react for 90 min at room temperature. After the reaction the electrode was rinsed with 100 mM phosphate buffer pH 7.1 and taken to the photoelectrochemical cell for the measurements.

***Trametes hirsuta* laccase covalent immobilization on bare FTO electrodes.** The process of functionalization and laccase attachment was equivalent to that described for FTO- In_2S_3 electrodes but using clean unmodified FTO.

***Trametes hirsuta* laccase physical adsorption on FTO- In_2S_3 electrodes.** 15 μL of ThLc solution was deposited on the FTO- In_2S_3 electrode surface and let to adsorb for 120 min.

Preparation of FTO-In₂S₃-CuSO₄ electrodes. 10 μL of an aqueous solution containing dissolved CuSO₄ (3 $\text{mg}\cdot\text{L}^{-1}$) were deposited on the FTO-In₂S₃ surface and let to dry.

Electrochemical measurements. A three-electrode cell with an Ag/AgCl (3M NaCl) reference electrode and a Pt wire counter electrode was used. The electrochemical cell was under Ar atmosphere and was surrounded by a refrigeration glass jacket connected to a thermostatic water recirculation system set at 30°C. For all the electrochemical measurements the cell was filled with 30 mL of phosphate buffer 100 mM at pH 7.1. The electrochemical measurements were controlled by a PG30 Autolab Potentiostat/Galvanostat from Ecochemie. The current density values are calculated considering the geometrical area of the electrodes (0.196 cm^2). All electrochemical measurements have been repeated at least 4 times; the shown CVs and chronamperometries correspond to typical results.

Illumination was carried out with a LOT Quantum Design 150W Xenon Lamp setting the output power at 8.5A. The light source was placed at 28 cm from the working electrode. UV radiation was filtered with either a 1 mm thick polycarbonate layer or optical filters from Andover Corporation: 450 nm band-pass filter, $\lambda \geq 400$ and $\lambda \geq 495$ cutoff filters. Light intensity was measured with a LP471 UVA Probe (315-400 nm) and with a LP471 RAD Probe (400-1050 nm). 10 measurements were averaged both in front and behind the electrochemical cell (without the electrode present) for each case.

The O₂ produced in the electrochemical cell was monitored with a low range oxygen microsensor from Unisense (Oxy Meter OX-NP-17180) placed at ca. 1.5 mm distance from the working electrode. It should be noted that working electrodes were measured only once each, afterwards they were disposed. All the results exposed have been performed at least 3 times, yielding the average values expressed in the tables.

H₂O₂ determination. The spectrophotometric determination of H₂O₂ was performed with a UV-2401 PC SHIMADZU spectrophotometer at $\lambda = 414$ nm following a known procedure.⁶⁵ The measurements were carried out after mixing in a cuvette 890 μL of sample, 100 μL of 9.1 mM ABTS and 10 μL of 1 $\text{mg}\cdot\text{mL}^{-1}$ HRP and letting to react during 8 min.

Trametes hirsuta laccase activity characterization. Laccase activity was determined by following ABTS oxidation at $\lambda = 414$ nm. The cuvette contained 2.25 mL of 100 mM phosphate buffer pH 4.4, 250 μL of 9.1 mM ABTS and the sample. For laccase in solution, increasing amounts of laccase were added to the cuvette. For electrode-attached laccase, the electrode was immersed in a cuvette side that did not interfere with the light path. For electrolyte-leaked laccase determination the electrolyte solution was concentrated by 3 cycles of centrifugation at 4000g using Centricon 3K filters. The concentrated solution was taken to 5 mL of 100 mM phosphate buffer pH 4.4. 2.5 mL of the concentrate were used for UV-Vis determination with the proper amount of ABTS.

Results

The synthesized polycrystalline $\beta\text{-In}_2\text{S}_3$ showed an orange-red color and its characterization has been reported previously.⁶² The particles ranged from 50 nm to micron-size agglomerates as observed by SEM, had a 37.2 nm crystal domain size determined by XRD, a 2.1 eV band gap, a specific area of $40.6 \pm 0.3 \text{ m}^2 \text{ g}^{-1}$, a total pore volume of $0.168 \text{ cm}^3 \text{ g}^{-1}$, and an average pore width of 16.5 nm.⁶²

Additional characterization was done after modification of the FTO electrodes with In₂S₃ particles. A clean sample of FTO, a FTO-In₂S₃ electrode, and a FTO-In₂S₃-ThLc electrode were visualized by SEM, Figure 2. As a result of the In₂S₃ deposition on the FTO surface the roughness increased. A more dense coverage of FTO with In₂S₃ was not tried to avoid multilayer coverage, which would hinder further electron transfer to the laccase. The further modification with the laccase did not yield any significant change at this scale, as expected, taking into account the much smaller size of laccase molecules⁶⁶ than that of the In₂S₃ particles.

Figure 2. SEM images of (a) bare FTO, (b) FTO In₂S₃, (c) FTO-In₂S₃-ThLc electrodes.

Figure 3 CVs of: bare FTO electrode dark (1a) and illuminated (1b); FTO-In₂S₃ electrode dark (2a) and illuminated (2b); FTO-In₂S₃ electrode with physically adsorbed ThLc dark (3a) and illuminated (3b). Scan rate was 20 mV/s.

Figure 4. CVs of: FTO electrodes modified with covalently bound ThLc measured in dark (1a) and under illumination (1b); FTO modified with In₂S₃ and functionalized with amino phenyl groups dark (2a) and illuminated (2b); FTO modified with In₂S₃ and ThLc dark (3a) and illuminated (3b). Scan rate was 20 mV/s.

Inset: Potentiometric signal recorded by the oxygen sensor while scanning 3a and 3b CVs.

XRD diffractograms were measured for the electrodes at different building stages: an FTO electrode, FTO-In₂S₃ electrode before and after 1 V voltage vs. SHE was applied during 360 s, and In₂S₃ before its addition to FTO, Figure S1. The peaks that characterize either FTO or In₂S₃ remain unchanged in their 2 θ values in all cases, although their intensity varies. The diffractograms also show that new diffraction peaks do not appear after application of 1 V to FTO-In₂S₃ electrodes that could be attributed to the appearance of oxysulfides or any other possible oxidation product.

The electrical response of FTO, FTO-In₂S₃ and FTO-In₂S₃-ThLc electrodes was followed by cyclic voltammetry under dark and illumination conditions. On the one hand, the FTO-In₂S₃-ThLc electrodes, on which the laccase was physically deposited, did not increase the electrochemical response provided by a FTO-In₂S₃ electrode under illumination, Figure 3. On the other hand, the FTO-In₂S₃-ThLc electrodes, on which the enzyme was covalently immobilized, behaved different, Figure 4. For potentials higher than 0.8 V vs SHE both bare FTO and FTO-In₂S₃ gave a current increase when illuminating the electrodes much smaller than the FTO-In₂S₃-ThLc ones, which increased more than 10-fold when illuminated (Figure 4). The O₂ microsensor placed ca. 1.5 mm away from the photoelectroactive surface detected O₂ produced by the FTO-In₂S₃-ThLc electrode only when it was illuminated, Figure 4 inset. These results indicate that covalent attachment of ThLc on the semiconductor surface is needed to oxidize H₂O to O₂.

The detection of O₂ by the sensor started as the backwards scan of the CV was initiated, suggesting that O₂ production by the illuminated FTO-In₂S₃-ThLc electrode took place at the higher potentials. A delay in the sensor signal is expected due to the diffusion time needed for the photobioelectrochemically produced O₂ to diffuse towards the sensor.

In order to determine at which redox potential O₂ started its evolution at the FTO-In₂S₃-ThLc electrode and to increase the magnitude of its detection, 5 min chronoamperometric measurements under dark and illumination conditions in presence of the O₂ sensor was performed, Figure 5. A typical FTO-In₂S₃ electrode provided a photocurrent increase due to illumination that yielded 0.262 mC charge at 1 V over 150 s, although the O₂ sensor did not give any significant signal change during the event, Figure 5A. The FTO-In₂S₃-ThLc electrode behaved differently, viz. the photocurrent increase at 1 V was more intense, yielding 0.496 mC over nearly the same time period, while the O₂ detected was significant. These results support the previous ones showing that covalent attachment of ThLc on the semiconductor surface is needed to oxidize H₂O to O₂.

Although the O₂ sensor provides evidence of H₂O oxidation catalyzed by a photoelectroenzymatic process, there are many factors hindering the quantification of the O₂ produced, specifically: i) the O₂ present in the solution is not homogeneously distributed during the experimental timescale, as it is produced in the electrode surface and the solution is quiescent; ii) not all the O₂ produced diffuses to or through the sensor; iii) the O₂ in solution equilibrates with the gas phase. All these reasons explain why only transient O₂ signals were measured during chronoamperometric studies, as well as the delay of approximately 30 s for the onset detection. The signal delay was attributed to O₂ diffusion limitations (supporting information). A correlation could be established between the O₂ produced by

an equivalent FTO surface at different overpotentials where H₂O oxidation takes place and the O₂ signal detected by the sensor when located exactly at the same position with respect to the electrode as in the photoelectrochemical measurements. The correlation was based on the results shown in Figures S2 and S3A, which indicate that the integration of the sensor signal over time correlates linearly with the charge measured during the associated FTO chronoamperometric experiments. On the other hand, the extent of H₂O oxidation to the byproduct H₂O₂ by the FTO electrodes biased at different potentials was also analyzed. An aliquot of the resulting electrolyte was taken and HRP and ABTS were added to it for H₂O₂ determination. The negligible ABTS oxidation detected spectrophotometrically at 414 nm implied a negligible H₂O₂ formation at any applied potential (Figure S3B), and consequently made clear that all charge was used essentially to produce O₂.

Figure 5. (A) Chronoamperogram recorded at 1 V vs. SHE on an FTO-In₂S₃ electrode (a), illuminating from 1.5 to 4 min. (b) O₂ signal measured by the sensor. (B) Chronoamperometry recorded at 1 V vs SHE on an FTO-In₂S₃-ThLc electrode (a), illuminating from 1.8 to 4 min. (b) O₂ signal measured by the sensor.

The calibration performed allowed studying the O₂ produced by a FTO-In₂S₃-ThLc electrode when illuminated during 6 min at different bias potentials. A bias potential of 0.7 V vs. SHE yielded no measurable O₂ signal, whereas in the case of 0.8-1.0 V potential range the amount of O₂ produced by the electrode could be estimated (Table 1). The current increase at

higher overpotential provided a higher and more reproducible O_2 production. A faradaic yield of $45 \pm 5\%$ is obtained at 1 V from the ratio between the amount of O_2 produced and the charge measured at the electrode during the chronoamperometry under light illumination. With the same calibration the amount of O_2 produced in the experiment of Fig. 5B was equivalent to 0.22 mC, which means that, in comparison with the result in Fig. 5A, the additional charge transferred in the presence of the enzyme (0.23 mC) corresponds almost completely to water oxidation to O_2 .

A control experiment was done with ThLc covalently bound to the functionalized FTO to evaluate at which redox potential the enzyme was able to evolve O_2 without photochemical excitation. Without illumination the O_2 production was not detected until 1.55 V was applied at the electrode (Fig. S4), which was the same potential required for measuring non-biocatalytic water oxidation directly at the bare FTO electrode (Figure S2)

Table 1. Charge and amount of O_2 produced by illuminated FTO-In $_2$ S $_3$ -ThLc electrodes under different applied potentials during 6 min. Each measure was recorded with a freshly prepared electrode.

E (SHE) / V	Charge (mC)	O_2 (nmol)	Faradaic yield (%)
0.7	0.3 ± 0.1	0	0
0.8	0.7 ± 0.1	0.5 ± 0.1	30 ± 5
0.9	0.6 ± 0.2	0.44 ± 0.03	30 ± 10
1.0	0.8 ± 0.2	0.93 ± 0.04	45 ± 5

The amount of active laccase immobilized on the FTO-In $_2$ S $_3$ -ThLc electrodes was estimated by the standard ABTS oxidation assay monitored by UV-VIS spectroscopy, which allows determining the amount of active enzyme from the rate of ABTS oxidation (in the presence of O_2).⁶⁰ First a calibration with different free ThLc concentrations ranging from 2.3 ng/mL to 140 ng/mL was performed (Figure S5), then different modified electrodes were placed in the side of an UV-VIS cuvette, and their ABTS oxidation activity was tested, Figure 6. The ThLc remained active after its covalent immobilization on either sulfide-loaded or FTO electrodes (Figure 6a and 6b). The average amount of the active enzyme on 4 different FTO-In $_2$ S $_3$ -ThLc electrodes was measured in this way from the UV-Vis spectroscopy data of the oxidized ABTS and found to be $0.18 \pm 0.08 \mu\text{g}$. However, after being simultaneously exposed to a positive potential and illumination, its activity dropped drastically, losing 99% of the initial value (Figure 6d and e). A sample from the electrolyte solution was measured to check if ThLc leaked during the operation. No active enzyme was detected in the solution (Figure 6c).

Figure 6 ABTS oxidation kinetics catalyzed by ThLc immobilized on FTO electrodes. (a) Freshly prepared FTO-ThLc electrode. (b) Freshly prepared FTO-In $_2$ S $_3$ -ThLc electrode. (c) Test made to detect any enzyme leaked into solution from FTO-In $_2$ S $_3$ -ThLc electrode after 6 min of photochronoamperometry at 0.8 V vs. SHE. (d) FTO-ThLc electrode after 6 min photochronoamperometry at 0.8 V vs. SHE. (e) FTO-In $_2$ S $_3$ -ThLc electrode after 6 min photochronoamperometry at 0.8 V vs. SHE. (f) FTO-ThLc electrode illuminated during 6 min with no applied potential. ABTS⁺ concentration was measured spectrophotometrically at 414 nm.

The ThLc stability against illumination was also measured by illuminating during 6 min in open circuit conditions a FTO/ThLc electrode. The average enzyme activity after the illumination was $7.5 \pm 0.1 \text{ ng}$ (Figure 6f), which is comparable to the activity measured on the electrodes after the photobioelectrochemical experiments. These control experiments allow attributing the laccase inactivation to the Xe lamp illumination.

Additional control experiments were performed to determine the enzyme sensitivity to the lamp illumination. The activity in open circuit conditions of FTO-ThLc after illumination with the Xe lamp through three filters ($\lambda \geq 400 \text{ nm}$, $\lambda \geq 495 \text{ nm}$, $420 \leq \lambda \leq 480 \text{ nm}$) was measured, finding out that the ABTS oxidation activity was reduced to 10%, 17% and 30%, respectively (Figure S6). The photoelectrochemical response and the O_2 production was also measured for these filtered samples, registering O_2 only in the case of $\lambda \geq 400 \text{ nm}$ allowed light. The light intensity was measured for all the exposed filtering configurations (Table S1). An approximate quantum yield of 0.5% was calculated for the case of FTO-In $_2$ S $_3$ -ThLc illumination with λ centered at 450 nm. The UV-Vis transmittance of the polycarbonate, band and cut-off filters used was measured, Figure S7.

Copper has been described as an O_2 evolution electrocatalyst^{5,67}. Therefore we considered that an undesired enzyme denaturalization might take place that releases from its active site Cu cations, which might adsorb on In $_2$ S $_3$ and lead to O_2 evolution. Three control experiments adding to FTO-In $_2$ S $_3$ electrodes instead of laccase an amount of CuSO $_4$ equivalent to the amount of Cu in the laccase yielded no O_2 detection upon illumination and 1.0V vs SHE applied voltage, Figure S8. This control hints that the laccase structure, not Cu ions formed by its photodecomposition, is involved in the O_2 evolution obtained with FTO-In $_2$ S $_3$ -ThLc electrodes.

Discussion

The formation of hybrid inorganic/biochemical photoelectrocatalysts by attachment of enzymes to semiconductor materials based electrodes has been shown for only few redox metalloenzymes, such as a hydrogenase⁶⁸ or a CO dehydrogenase.⁶⁹ Adsorption of a laccase to p-type silicon on a gold electrode has been reported for photoelectrochemical reduction of O₂.⁷⁰ However, the photoelectrocatalytic properties of the hybrid device were considerably worse than those obtained with high redox potential laccases directly attached to electrodes⁴¹, which give high catalytic currents of O₂ bioelectroreduction at low overpotentials accompanied with a high operational stability.⁴⁴⁻⁴⁹

Therefore, it is of much interest to develop laccase/semiconductor hybrids as photoelectrocatalysts devices for the reverse reaction, i.e. water oxidation to O₂. This process still requires the development of efficient catalysts based on cheap metals. The use of copper complexes as electrochemical catalysts for H₂O oxidation has been proven,^{67,54} as has the use of copper-containing redox enzymes such as laccase^{41,43,44} and bilirubin oxidase.^{44,50} Although the overpotential shown by copper complexes is very promising, the use of an extra energy provided by the light may help to reduce the energy to invest in the process. The In₂S₃ semiconductor is able to absorb visible light, and has shown ability to transfer the light-excited electrons to a hydrogenase enzyme in presence of a sacrificial electron donor so that the enzyme reduces protons to H₂.⁶² In₂S₃ may be also coupled to electrodes to promote oxidative reactions, as the excited electrons can be swiftly transferred to the positively polarized electrode before recombination.⁵³ Hence, immobilizing the laccase on the surface of the In₂S₃ modified electrode can take the advantage of the light energy to reduce the applied potential needed for H₂O electrooxidation. Taking into account that the valence band level of In₂S₃ lies below the standard potential for H₂O oxidation⁵², the photogenerated hole is suitable to accept the electrons from H₂O molecules, if coupled with an adequate catalyst.

The O₂ production by the FTO-In₂S₃-ThLc system requires a lower electric energy input thanks to the light assistance. The onset potential for the process is 0.8 V vs. SHE at pH 7.1, which corresponds to 1.24 V vs. RHE. Therefore, the hybrid photoelectrocatalyst is able to oxidize water to O₂ with 30% faradaic efficiency at practically the theoretical thermodynamic potential value; it should be noted that the theoretical potential value considers P(O₂) = 1 Atm, and we are measuring at much lower partial pressure, specialty at t = 0. The same laccase attached to porous graphite electrode did not produce O₂ below 1.2 V vs. SHE at the same pH,⁴¹ thus the photoelectrochemical strategy allows the reduction of the overpotential for H₂O electrooxidation by at least 0.4 V. When the enzyme is covalently bound directly to an FTO electrode, O₂ production is negligible below the redox potential, at which water is oxidized directly at the FTO (1.55 V). This result can be explained by the hindrance of establishing direct electron transfer of the laccase at a less rough and conductive electrode than low density graphite.⁴¹ Instead, the porous surface of the In₂S₃ favors the electron transfer to the redox sites of the attached laccase molecules under light illumination, as has been previously observed for another enzyme, hydrogenase.⁶² In the present case, covalent binding of the enzyme to the functionalized semiconductor was required, possibly because the smaller size of the laccase molecules than the hydrogenase ones pre-

vents their retention in the In₂S₃ pores just by adsorption. Another possible explanation is that hydrogenase was stabilized by S-S attracting interactions between the chalcogenide's surface and the distal cluster of the enzyme,⁶² whereas in this work laccase lacks any surface motif to promote a strong and oriented adhesion by simple non-covalent adsorption.

As derived from the results with filtered light it seems that not all the photons able to promote electrons to the conduction band generate holes with such a low energy to allow the O₂ evolution catalyzed by the laccase. Despite that the light absorption onset for In₂S₃ is $\lambda \leq 600$ nm,⁶² it was needed to allow the photons with $\lambda \geq 400$ nm to obtain a measurable O₂ production (Fig. S6). Photons with $\lambda \geq 495$ nm did not drive O₂ evolution although the light power reaching the FTO-ThLc electrode was high enough (Table SI). A possible explanation is that higher energy photons are able to generate hot carriers, which may be the responsible of promoting the photobiocatalytic process. The fact that the amount of O₂ generated using the $420 \leq \lambda \leq 480$ nm filter is much smaller than that found with the $\lambda \geq 400$ nm filter (Figs. S6D and S6B respectively) may be due to the significantly lower amount of photons hitting the electrode in the first case (see Fig. S7).

The faradaic yield of the FTO-In₂S₃-ThLc device increases to 45%, when the electrode is polarized at 1 V, which is comparable to the faradaic efficiencies reported for some inorganic photoelectrocatalysts for O₂ production.⁷¹ The photocurrent measured at 1 V in the control FTO-In₂S₃ electrode without laccase is approximately 53% of that for the FTO-In₂S₃-ThLc electrode (Fig. 5), suggesting that the main cause that decreases the faradaic yield of the later system for O₂ production is the photocorrosion of the In₂S₃.⁵³ Still, we can note that the amount of charge transferred in the experiment of Fig. 5A, considering the amount of In₂S₃ on the electrode active area (~ 20 μ g) and the specific surface of the material (≈ 40 m²/g), is equivalent to ≈ 2.0 holes/nm² part of which might be accumulated on the surface by a capacitance effect, not by a redox process; the extent of sulphide photocorrosion in these experiments must be therefore limited. Detection of such low photocorrosion rates is beyond the detection limits of most analytical techniques.

A benchmark for biophotoelectrocatalytic O₂ production has been reported by Reisner and co-workers using photosystem II (PSII) immobilized within a Os complex redox polymer on nanostructured ITO electrodes.³⁷ High photocurrents up to 410 μ A cm⁻² at 0.5 V vs. SHE have been reported with 85% faradaic efficiency. However, the photocurrents measured with PSII decrease two orders of magnitude, when working in direct electron transfer mode, i.e. in the absence of the redox polymer that shuttles electrons between the redox centers of PSII and the electrode.⁴⁰ In our system we measure photocurrents up to 50 μ A cm⁻² (at 1 V) in direct electron transfer mode. Considering the determined amount of active laccase immobilized on the photoanode, its turnover frequency for O₂-production is on average 4.6 ± 0.1 s⁻¹, which equals that measured for PSII.³⁷ Therefore, high photocurrents as those measured with PSII could be obtained, if the enzyme coverage was increased by using a nanostructured ITO electrode instead of the planar FTO electrodes and/or the FTO coverage with the In₂S₃ were more homogeneous. Indeed, PSII is able to evolve O₂ with the same turnover frequency at 0.5 V lower potential, but it has to be taken into account that PSII is nature's evolved catalyst for water oxidation, whereas the natural activity of

laccases is the reverse reaction. Thus, adequate design of the photoelectrocatalytic device allows driving the enzymatic reaction with the aid of light to the desired direction, even if this reaction is not the thermodynamically or kinetically favored one under the standard conditions for this enzyme.

One of the main concerns for the solar system including biodevices is the photocurrent decrease with time, which could originate from different sources, such as degradation of the sulfide, the enzyme, or any of the interfaces. The surface of FTO is stable enough, so it can be excluded as cause of the low operational stability. Monitoring of the XRD pattern of In_2S_3 before and after the photochemical water oxidation process gave no hint of indium traces of oxysulfides or oxides. The specific native activity of the biocatalyst was reduced by a factor of 99% after 6 min operation under illumination, either attached to the electrode or free in solution. This inactivation may be attributed (i) to the attack of the hydroxyl radicals formed from the photogenerated holes⁷² to the copper cations at the enzyme's active site, which blocks the enzyme and/or (ii) to the photodegradation of the enzyme under high power illumination. The second explanation is in agreement with our experimental result shown in Figure 6f, where a freshly prepared electrode FTO-ThLc lost any O_2 reducing/ABTS oxidizing catalytic activity after illumination, even if it was never connected to the electrochemical setup. We tested the system illuminated with filtered light, finding out that damage to laccase molecules occurred in the whole visible range and it increased with the light intensity. Nevertheless, it may be possible that the O_2 -evolution activity of the laccase under irradiation is less unstable than its O_2 -reduction activity with ABTS as substrate, as previous electrocatalytic studies suggested that the Cu T1 site (where ABTS reacts with the enzyme) is not involved in the former activity.⁴¹

Conclusions

ThLc has been successfully presented as catalyst for O_2 evolution in combination with In_2S_3 particles deposit on FTO transparent electrode to assemble a light-assisted photoelectrochemical anode. The turnover frequency per enzyme molecule given by the FTO- In_2S_3 -ThLc system is comparable to the best-reported photobioelectrochemical system,³⁷ opening a route for future much-needed improvement. Such improvement may come by using nanostructured ITO electrodes for obtaining higher enzyme coverage and by obtaining mutant laccases with higher operational stability under light illumination and/or turnover towards O_2 evolution. Directed evolution has shown previously to be a successful strategy to obtain mutated laccases with enhanced properties and activity in harder environments, such as neutral pH or organic solvents,^{73,74} and may yield more appropriate biocatalysts. The use of intermediate-band semiconductors may also improve the fraction of the solar energy spectrum able to drive the photo-induced chemical change, allowing low-energy photons to add their energy and pump electrons to the conductive band.^{53,63}

ASSOCIATED CONTENT

Supporting Information

XRD characterization of modified electrodes, FTO non-catalytic O_2 evolution measurements, O_2 evolution calibrations, and exper-

iments with filtered light are included as Supporting Information. The Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Author

* Marcos Pita: marcospita@icp.csic.es; Antonio L. De Lacey: alopez@icp.csic.es. Instituto de Catalisis y Petroleoquímica, CSIC. C/ Marie Curie, 2. L10 28049 Madrid, Spain.

Funding Sources

This work was funded by the Spanish MINECO/FEDER projects CTQ2015-71290-R and ENE2013-46624-C4-1-R, the CSIC PIE 201560I018 project and by the European Commission project "Bioenergy" FP7-PEOPLE-2013-ITN-607793. C.T. scholarship is funded by the Spanish MINECO contract BES-2013-064099.

REFERENCES

- (1) Fujishima, A.; Honda, K. *Bull. Chem. Soc. Japan* **1971**, *44*, 1148-1150.
- (2) Fujishima, A.; Honda, K. *Nature* **1972**, *238*, 37.
- (3) Li, X.; Yu, J.; Low, J.; Fang, Y.; Xiao, J.; Chen, X. *J. Mater. Chem. A* **2015**, *3*, 2485-2534.
- (4) Lewis, N. S.; Nocera, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15729-15735.
- (5) House, R. L.; Iha, N. Y. M.; Coppo, R. L.; Alibabaei, L.; Sherman, B. D.; Kang, P.; Brennaman, M. K.; Hoertz, P. G.; Meyer, T. J. *J. Photochem. Photobiol. C: Photochem. Rev.* **2015**, *25*, 32-45.
- (6) Lewis, N. S. *Science* **2016**, *351*, 353.
- (7) White, J. L.; Baruch, M. F.; Pander III, J. E.; Hu, Y.; Fortmeyer, I. C.; Park, J. E.; Zhang, T.; Liao, K.; Gu, J.; Yan, Y. *et al. Chem. Rev.* **2015**, *115*, 12888-12935.
- (8) Beck, F.; Gerischer, H. *Z. Elektrochem.* **1959**, *63*, 943-950.
- (9) Green, M. *Modern Aspects of Electrochemistry* **1959**, *2*, 343-407.
- (10) Gerischer, H. *J. Electrochem. Soc.* **1966**, *113*, 1174.
- (11) Lohmann, F. *Ber. Bunsenges. Phys. Chem.* **1966**, *70*, 87-92.
- (12) Renz, C. *Helv. Chim. Acta* **1921**, *4*, 961-968.
- (13) Yamamoto, M.; Tanaka, K. *ChemPlusChem* **2016**, *81*, 1028-1044.
- (14) Sprick, R.S.; Jiang, J.-X.; Bonillo, B.; Ren, S.; Ratvijitvech, T.; Guiglion, P.; Zwijnenburg, M.A.; Adams, D.J.; Cooper, A.I. *J. Am. Chem. Soc.* **2015**, *137*, 3265-3270.
- (15) Amao, Y. *ChemCatChem* **2011**, *3*, 458-474.
- (16) Babu, V.J.; Vempati, S.; Uyar, T.; Ramakrishna, S. *Phys. Chem. Chem. Phys.* **2015**, *17*, 2960-2986.
- (17) Kubacka, A.; Fernández-García, M.; Colón, G. *Chem. Rev.* **2012**, *112*, 1555-1614.
- (18) Sivula, K.; Van de Krol, R. *Nat. Rev. Materials* **2016**, *1*, 15010.
- (19) Trasatti, S. *J. Electroanal. Chem.* **1980**, *111*, 125-131.
- (20) Lee, Y.; Suntivich, J.; May, K. J.; Perry, E. E.; Shao-Horn, Y. *J. Phys. Chem. Lett.* **2012**, *3*, 399-404.
- (21) Walter, M. G.; Warren, E. L.; McKone, J. R.; Boettcher, S. W.; Mi, Q.; Santori, E. A.; Lewis, N. S. *Chem. Rev.* **2010**, *110*, 6446-6473.
- (22) McCrory, C. C. L.; Juong, S.; Peters, J. C.; Jaramillo, T.F. *J. Am. Chem. Soc.* **2013**, *135*, 16977-16987.
- (23) Matheu, R.; Francas, L.; Chernev, P.; Ertem, M. Z.; Batista, V.S.; Haumann, M.; Sala, X.; Llobet, A. *ACS Catal.* **2015**, *5*, 3422-3429.
- (24) Doyle, R. L.; Godwin, I. J.; Brandon, M. P.; Lyons, M. E. G. *Phys. Chem. Chem. Phys.* **2013**, *15*, 13737-13783.
- (25) Corrigan, D. A. *J. Electrochem. Soc.* **1987**, *134*, 377-384.
- (26) Trotochaud, L.; Young, S. L.; Ranney, J. K.; Boettcher, S. W. *J. Am. Chem. Soc.* **2014**, *136*, 6744-6753.

- (27) Zhang, B.; Zheng, X.; Voznyy, O.; Comin, R.; Bajdich, M.; Garcia-Melchor, M.; Han, L.; Xu, J.; Liu, M.; Zheng, L. *et al. Science* **2016**, *352*, 333-337.
- (28) Kanan, M. W.; Nocera, D. G. *Science* **2008**, *321*, 1072-1075.
- (29) Soriano-López, J.; Goberna-Ferroón, S.; Vígara, L.; Carbó, J. J.; Poblet, J.M.; Galán-Mascarós, J.R. *Inorg. Chem.* **2013**, *52*, 4753-4755
- (30) Najafpour, M.M.; Renger, G.; Holyńska, M.; Moghaddam, A.N.; Aro, E.M.; Carpentier, R.; Nishihara, H.; Eaton-Rye, J.J.; Shen, J.R.; Allakhverdiev, S.I. *Chem. Rev.* **2016**, *116*, 2886-2936.
- (31) Sun, L. *Science* **2015**, *348*, 635-636.
- (32) Du, J.; Chen, Z.; Ye, S.; Wiley, B.J.; Meyer, T.J. *Angew. Chem. Int. Ed.* **2015**, *54*, 2073-2078.
- (33) Garrido-Barros, P.; Funes-Ardoiz, I.; Drouet, S.; Benet-Buchholz, J.; Heberle, J.; Maseras, F.; Lobet, A. *J. Am. Chem. Soc.* **2015**, *137*, 6758-6761.
- (34) Najafpour, M.; Ghobadi, M.; Larkum, A.; Shen, J.; Allakhverdiev, S. *Trends Plant Sci.* **2015**, *20*, 559-568.
- (35) Badura, A.; Esper, B.; Ataka, K.; Grunwald, C.; Woll, C.; Kuhlmann, J.; Heberle, J.; Rogner, M. *Photochem. Photobiol.* **2006**, *82*, 1385-1390.
- (36) Yehezkeili, O.; Tel-Vered, R.; Wasserman, J.; Trifonov, A.; Michaeli, D.; Nechushtai, R.; Willner, I. *Nat. Commun* **2012**, *3*, 742.
- (37) Sokol, K.; Mersch, D.; Hartmann, V.; Zhang, J.; Nowaczyk, M.; Rogner, M.; Ruff, A.; Schuhmann, W.; Plumere, N.; Reisner, E. *Energy Environ. Sci.* **2016**, *9*, 3698-3709.
- (38) Alonso-Lomillo, M. A.; Rudiger, O.; Maroto-Valiente, A.; Velez, M.; Rodriguez-Ramos, I.; Munoz, F. J.; Fernandez, V. M.; De Lacey, A. L. *Nano Lett.* **2007**, *7*, 1603-1608.
- (39) Leger, C.; Bertrand, P. *Chem. Rev.* **2008**, *108*, 2379-2438.
- (40) Kato, M.; Cardona, T.; Rutherford, A.; Reisner, E. *J. Am. Chem. Soc.* **2013**, *135*, 10610-10613.
- (41) Pita, M.; Mate, D. M.; Gonzalez-Perez, D.; Shleev, S.; Fernandez, V. M.; Alcalde, M.; De Lacey, A. L. *J. Am. Chem. Soc.* **2014**, *136*, 5892-5895.
- (42) Solomon, E. I.; Augustine, A. J.; Yoon, J. *Dalton Trans.* **2008**, 3921-3932.
- (43) Shleev, S.; Jarosz-Wilkolazka, A.; Khalunina, A.; Morozova, O.; Yaropolov, A.; Ruzgas, T.; Gorton, L. *Bioelectrochemistry* **2005**, *67*, 115-124.
- (44) Tsujimura, S.; Kamitaka, Y.; Kano, K. *Fuel Cells* **2007**, *7*, 463-469.
- (45) Blanford, C. F.; Foster, C. E.; Heath, R. S.; Armstrong, F. A. *Faraday Discuss.* **2008**, *140*, 319-335.
- (46) Shleev, S.; Shumakovich, G.; Morozova, O.; Yaropolov, A. *Fuel Cells* **2010**, *10*, 726-733.
- (47) Parimi, N. S.; Umasankar, Y.; Atanassov, P.; Ramasamy, R. P. *ACS Catal.* **2012**, *2*, 38-44.
- (48) Lalaoui, N.; Elouarzaki, K.; Le Goff, A.; Holzinger, M.; Cosnier, S. *Chem. Commun.* **2013**, *49*, 9281-9283.
- (49) Di Bari, C.; Goni-Urtiaga, A.; Pita, M.; Shleev, S.; Toscano, M. D.; Sainz, R.; De Lacey, A. L. *Electrochim. Acta* **2016**, *191*, 500-509.
- (50) Shleev, S.; Andoralov, V.; Pankatov, D.; Falk, M.; Aleksejeva, O.; Blum, S. *Electroanalysis* **2016**, *28*, 2270-2287.
- (51) Becker, R. S.; Zheng, T.; Elton, J.; Saeki, M. *Sol. Energ. Mat.* **1986**, *13*, 97-107.
- (52) Xu, Y.; Schoonen, M. A. A. *Am. Mineralogist* **2000**, *85*, 543-556.
- (53) Tapia, C.; Berglund, S.P.; Friedrich, D.; Ditttrich, T.; Bogdanoff, P.; Liu, Y.; Levchenko, S.; Unold, T.; Conesa, J. C.; De Lacey, A.L.; Pita, M.; Fiechter, S. *J. Phys. Chem. C* **2016**, *120*, 28753-28761.
- (54) An, L.; Zhou, P.; Yin, J.; Chen, F.; Liu, H.; Du, Y.; Xi, P. *Inorg. Chem.* **2015**, *54*, 3281-3289.
- (55) Tian, Y.; Wang, L.; Tang, H.; Zhou, W. *J. Mater. Chem. A* **2015**, *3*, 11294-11301.
- (56) Braiek, Z.; Brayek, A.; Ghoul, M.; Ben Taieb, S.; Gannouni, M.; Ben Assaker, I.; Souissi, A.; Chtourou, R. *J. Alloys Comp.* **2015**, *653*, 395-401.
- (57) Wang, L.; Xia, L.; Wu, Y.; Tian, Y. *ACS Sustainable Chem. Eng.* **2016**, *4*, 2606-2614
- (58) Becker, R.S.; Zheng, T.; Elton, J.; Saeki, M. *Solar Eng. Mater.* **1986**, *13*, 97-107.
- (59) Herero, J.; Ortega, J. *Solar Eng. Mater.* **1988**, *17*, 357-368.
- (60) Shleev, S.; Morozova, O.V.; Nikitina, O.V.; Gorshina, E.S.; Rusinova, T.V.; Serezhnikov, V.A.; Burbaev, D.S.; Gazaryan, I.G.; Yaropolov, A.I. *Biochimie* **2004**, *86*, 693-703.
- (61) Bradford, M.M. *Anal. Biochem.* **1976**, *72*, 248-254.
- (62) Tapia, C.; Zacarias, S.; Pereira, I. A. C.; Conesa, J. C.; Pita, M.; De Lacey, A. L. *ACS Catalysis*, **2016**, *6*, 5691-5698.
- (63) Lucena, R.; Aguilera, I.; Palacios, P.; Wahnón, P.; Conesa, J. C. *Chem. Mater.* **2008**, *20*, 5125-5127.
- (64) Allongue, P.; Delamar, M.; Desbat, B.; Fagebaume, O.; Hitmi, R.; Pinson, J.; Saveant, J. M. *J. Am. Chem. Soc.* **1997**, *119*, 201-207.
- (65) Pita, M.; Shleev, S.; Ruzgas, T.; Fernandez, V.M.; Yaropolov, A.I.; Gorton, L. *Electrochim. Commun.* **2006**, *8*, 747-753.
- (66) Plyakov, K. M.; Federova, T. V.; Stepanova, E. V.; Cherkashin, E. A.; Kurzeev, S. A.; Strokopytov, B. V.; Lamzin, V. S.; Koroleva, O. V. *Acta Crystallogr.* **2009**, *65*, 611-617.
- (67) Chen, Z.; Meyer, T. *Angew. Chem. Int. Ed.* **2013**, *52*, 700-703.
- (68) Bachmeier, A.; Wang, V. C. C.; Woolerton, T. W.; Bell, S.; Fontecilla-Camps, J. C.; Can, M.; Ragsdale, S. W.; Chaudhary, Y. S.; Armstrong, F. A. *J. Am. Chem. Soc.* **2013**, *135*, 15026-15032.
- (69) King, P. W. *Biochim. Biophys. Acta* **2013**, *1827*, 949-957.
- (70) Skorupska, K.; Lewerenz, H. J.; Berzal, P. U.; Rutkowska, I. A.; Kulesza, P. J. *J. Mater. Chem.* **2012**, *22*, 15267-15274.
- (71) Alibabaei, L.; Sherman, B.; Norris, M.; Brennaman, M.; Meyer, T. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5899-5902.
- (72) Lucena, R.; Fresno, F.; Conesa, J.C. *Catal. Commun.* **2012**, *20*, 1-5.
- (73) Zumarraga, M.; Bulter, T.; Shleev, S.; Polaina, J.; Martinez-Arias, A.; Plou, F.J.; Ballesteros, A.; Alcalde, M. *Chem. Biol.* **2007**, *14*, 1052-1064.
- (74) Mate, D.M.; Gonzalez-Perez, D.; Falk, M.; Kittl, R.; Pita, M.; De Lacey, A.L.; Ludwig, R.; Shleev, S.; Alcalde, M. *Chem. Biol.* **2013**, *20*, 223-231.

SYNOPSIS TOC