reproducible pH change to the microelectrode. Using $\mu = 0.1$ M pH 7.2 phosphate buffer in one reservoir and $\mu = 0.1$ M pH 3.9 acetate buffer in the other, we can cycle the pH of the solution flowing past the WO_3-coated microelectrode. The results of this experiment are shown in Figure 8, where $V_G = -0.5$ V vs. SCE, $V_D = 150$ mV, and the solvent flow rate is 6.0 mL/min. I_D is monitored over time and is found to be 0.08 μA for the pH 7.2 solution and 0.8 μA for the pH 3.9 solution. This I_D is less than what would be expected from Figure 5 but is consistent with the resistance data in Figure 3. The changeover from one pH to the other requires approximately 45 s; I_D reaches the steady-state value within 90 s of when the transistor is exposed to the new pH solution. The stream was continuously flowed past the microelectrode for 6 h, without any degradation in the response time or the steady-state I_D at either pH. Thus, a WO_3-based transistor can sense changes in pH and gives large, reproducible currents in real time. The magnitude of I_D is such that much smaller changes in pH should be detectable without further amplification of I_D.

Conclusions

The operation of pH-sensitive microelectrochemical transistors based on WO_3-connected microelectrodes has been demonstrated. The response time of these devices is slower and power amplification is smaller than for microelectrochemical transistors based on conducting organic polymers, but the prospect of major improvements through control of deposition conditions and microelectrode geometry is possible. Unlike previously characterized microelectrochemical devices, the conducting region is accessed by negative values of V_G. This work shows that oxide-based transistors can be durable and that, in principle, many oxides with widely varying properties can be used to fabricate microelectrochemical devices.

The effect of pH on the transistor behavior of WO_3-connected microelectrodes has been probed; agreement with predictions based on the pH dependence of the electrochemical reduction of WO_3 has been found. Real-time I_D response, at fixed V_G and V_D, to pH change in a flowing steam has been shown. The full range of I_D is achievable at pH ≈ 7 through control of V_G. The transconductance of these microelectrochemical transistors is sufficiently large to ensure significant I_D in the V_G range -0.25 to -0.8 V vs. SCE at pH ≈ 7, where the redox potential of many biological reducing agents are found, meaning WO_3-based devices may be of value in sensing biological molecules.

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Registry No. WO_3, 1314-35-8; Au, 7440-57-5; Pt, 7440-06-4.

Direct Photoconversion of Pyruvate to Lactate in Aqueous TiO_2 Dispersions

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Pyruvate is efficiently converted to lactate under illumination of aqueous suspensions of titanium dioxide powder. This photoconversion does not require additional catalysts and its efficiency depends on the pH and the electron donor present in solution. Other keto carboxylic acids can also be photoreduced by the same process. The kinetics of reduction has been studied by monitoring the interfacial electron transfer occurring at the surface of colloidal TiO_2 semiconducting particles using laser photolysis .

Introduction

Heterogeneous photocatalysis on semiconductor particulate systems has become an active area of research in photochemistry.[1] Most of the photoprocesses studied deal, however, mainly with oxidation reactions by valence band holes and/or hydrogen production by conduction band electrons. Few papers report on heterogeneous photosynthetic organic reactions such as amino acid synthesis[2,3] or photodehydrogenation.[4]

In the course of investigations on photoassisted enzymatic catalysis, we observed in the presence of electron donors the efficient reduction of pyruvate to lactate under illumination of suspensions of titanium dioxide powders in a sunlight simulator. The photoconversion occurs by direct reaction of pyruvate with conduction band electrons and does not require additional catalysts. Using colloidal semiconduction TiO_2 particles, we have applied laser photolysis to directly monitor the time course of the reaction. Our results confirm that this technique applied to finely dispersed colloidal suspensions is a powerful tool in the study of the dynamics of surface-catalyzed redox reactions.

Compared to other photochemical carbonyl-reducing systems such as the enzymatic pyruvate production via NAD(P)H photoregeneration[5] or the reduction of methyl benzoylformate in functionalized micelles,[6] TiO_2-assisted photosynthesis appears to be simple and efficient, although not stereospecific. Reduction of other keto carboxylic acids proved that the same catalytic mechanism could probably be applied to other organic photoprocesses of practical application.

Experimental Section

Lactate, pyruvate, and other carboxylic acids were analyzed on a Varian 5020 HPLC equiped with a AX-5 Micropak anion exchange column. The mobile phase was 20 mM KH_2PO_4, pH 3.4, and elution was checked by absorbance at 210 nm. Acetaldehyde and ethanol contents have been measured (GC) by Dr. P. Ammann, Institut de gênie biologique, EPFL. Light-induced reactions were assayed in 14-mL glass vials fitted with crimped septae containing 4 mL of TiO_2 suspensions. The vials were degassed for 15 min with argon and transferred to a sunlight simulator (Suntest, Hanau, Germany) fitted with a glass filter (cutoff at 320 nm) and delivering about 750 W m^{-2}. After irradiation suspensions were centrifuged in an Eppendorf mini-

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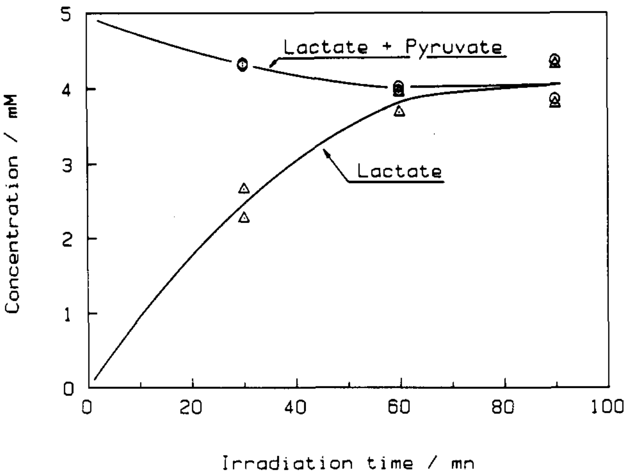

Figure 1. Lactate formation and lactate + pyruvate evolution vs. irradiation time in 0.5 g L^{-1} P25 TiO_2 containing 5 mM pyruvate and 50 mM TEOA (pH 7) initial concentrations.

centrifuge and supernatants analyzed by HPLC.

Enzymatic determination of L(+)-lactate was done according to Bergmeyer.[7] Pyruvate adsorption was determined by enzymatic measurement of pyruvate[7] in supernatants after centrifugation of TiO_2 suspensions containing the desired additives.

Degussa P25 TiO_2 (50 m^2 g^{-1}) was kindly provided by Dr. Kleinschmidt (Degussa AG, Hanau, F.R.G.) and Bayersol anatase (145 m^2 g^{-1}, doped with 600 ppm of Nb) was a gift of Dr. Panek, Bayer GmbH, F.R.G. Colloidal TiO_2 was prepared by Dr. J. Moser in our laboratory.[8] Laser photolysis employed a frequency-doubled JK 2000 ruby laser combined with a fast kinetic spectroscopy technique and a data aquisition system developed by Dr. P. P. Infelta. Colloidal suspensions were degassed with argon prior to the flash experiments. Pyruvate was purchased from Sigma and other chemicals from Fluka.

Results and Discussion

Pyruvate Photoreduction in TiO_2 Powder Suspensions. The time course of the pyruvate photoassisted reduction by P25 TiO_2 powder was monitored by measuring the concentrations of both lactate and pyruvate present in the suspensions at different irradiation times. Figure 1 shows the evolution of both lactate formed and the sum of lactate and pyruvate in the solution for samples containing 0.5 g L^{-1} of TiO_2, 50 mM TEOA as electron donor (pH 7), and an initial pyruvate concentration of 5 mM. Clearly, lactate is the main product evolved in the course of irradiation. Pyruvate is, however, converted by concurrent side reactions to some other products as indicated by the conversion efficiency measured at the end of the reaction, which shows that under the conditions reported here about 80% of the initial pyruvate has been reduced to lactate. The remaining 20% is probably transformed into products such as decarboxylated species, which were not further analyzed.

Control experiments showed that no lactate is formed in the absence of TiO_2 and that the reduction is a light-dependent process. Further evidence of the direct photoformation of lactate was obtained by measuring the enzymatic reoxidation of the compound present after total photoconversion of pyruvate. This control was performed by recording the reduction of NAD in the presence of L(+)-lactate dehydrogenase (EC 1.1.1.27) coupled to alanine-2-oxoglutarate aminotransferase to remove the pyruvate formed. Absorbance measurements at 340 nm showed that 50% of the product of the photoreaction was reoxidized by the stereospecific enzyme, thus confirming that the TiO_2-catalyzed reaction produces a racemic mixture of lactate.

The catalytic role of TiO_2 surfaces in the photoassisted pyruvate reduction was demonstrated by experiments run at low TiO_2 concentration. The final lactate concentration after 8 h of irradiation of a suspension containing 50 mg of TiO_2 L^{-1} in 40 mL of TEOA (50 mM) and pyruvate (5 mM), pH 7, was 4.3 mM (only traces of pyruvate left). Catalyst turnover is defined as the number of lactate molecules transformed per number of catalytic sites. Assuming that every OH group of the TiO_2 surface is catalytic, and using the hydroxyl density determined on the same TiO_2 material (470 μequiv OH groups per g of P25 TiO_2[9]), yield a turnover of 180, showing that photoassisted lactate production fulfills the conditions for a truly photocatalytic heterogeneous system.[10]

TABLE I: Rate of Lactate Production in the Presence of Different Donors at pH 9

	mmol lactate h^{-1} L^{-1}
no donor	1.2
50 mM TRIS	4.4
5% methanol	2.8
5% ethanol	2.2
50 mM EDTA	1.2
50 mM TEOA	3.8

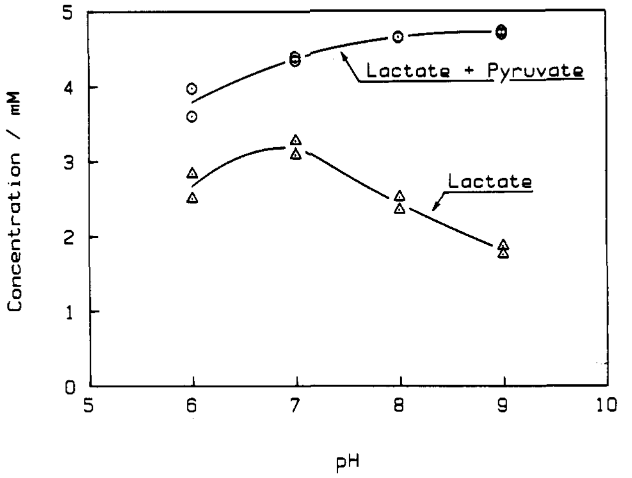

Figure 2. Lactate and lactate + pyruvate concentrations in TiO_2 suspensions as a function of pH after 60 min of irradiation (50 mM TEOA).

The production rate measured in the first 6 h of irradiation was 0.6 mM/h, corresponding to 11.8 mmol of lactate formed per g of TiO_2 per hour. (For a 10-fold higher TiO_2 concentration, a slightly lower rate is obtained (7.6 mmol of lactate g^{-1} of TiO_2 h^{-1}) due to light scattering and geometric factors of the irradiation setup.)

Electron Donor Efficiency. Various compounds have been tested as scavengers of the valance band holes produced under band gap excitation of TiO_2. As shown in Table I, where lactate production rates measured after 30 min of irradiation are reported, the photoreduction efficiency depends on the electron donor used. In the absence of sacrificial donor, lactate is still produced upon illumination. The analysis of both pyruvate and lactate concentrations showed however, that, without any donor, the rate of pyruvate disappearance is more than twice the rate of lactate formation (conversion efficiency lower than 50%). Here again, no other significant peaks are observed in the chromatogram showing that pyruvate decomposition is probably due to oxidative decarboxylation by valence band holes.

Further insights in the complex chemistry involved in the coupled photooxidation of the donor and reduction of pyruvate have been obtained by using triethanolamine as electron donor. Figure 2 shows the pH dependence of both lactate and lactate + pyruvate concentrations measured after 1 h of irradiation. It was verified that, due to the buffering capacity of the donor, the pH of the suspension did not vary significantly in the course of the experiment. It should be pointed out that, due to a different

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irradiation setup, the values reported in Figure 2 cannot be directly compared to the data showed in Figure 1.

The shape of the lactate production rate vs. pH can be rationalized by considering two independent processes. The decrease observed at higher pH is due to the conditions governing the interfacial charge-transfer reactions at the surface of the photocatalyst. Due to its amphoteric nature, TiO_2 is characterized by a surface potential which depends on the proton concentration at the interface. As the pH increases above the isoelectric point (which is around pH 6 for TiO_2 powders[11,12]) this potential becomes more negative. The electrostatic interactions between the surface of the photocatalyst and the carboxylic acid tend to decrease the interfacial concentration of pyruvate, which results in the observed decrease of the conversion efficiency of the process as the pH of the suspension increases.

Another effect explains the efficiency decrease observed at lower pH. As shown on the same figure, the sum of lactate produced and pyruvate remaining in the solution after irradiation decreases, indicating that upon irradiation a higher fraction of pyruvate is used in side reactions. This is due to the known decrease in the efficiency of TEOA oxidation at lower pH which favors the competing pyruvate oxidation by TiO_2 valence band holes. The use of other donors such as EDTA, which is known to be a better donor at more acidic pH, or MES has shown a higher lactate production rate at pH 5, thus confirming that the conversion rate depends strongly on the efficiency of valence band hole scavenging by sacrificial donors.

The effect of electrons donor concentration was also studied with TEOA (pH 7). A rapid increase in lactate production rate was noted up to 50 mM donor concentration, followed by a slight decrease up to 200 mM (data not shown). The measurement of the sum of lactate and pyruvate concentrations, effected in parallel, indicated that the pyruvate and/or lactate decomposition by side reactions is less efficient as the electron donor concentration increases, thus confirming the competition between TEOA and the carboxylic acids for the valence band holes formed in the semiconducting particles upon band gap irradiation.

Additional experiments were performed to elucidate further the two-electron-two-proton reduction of carbonyl groups at the TiO_2/solution interface. It was observed that lactate is evolved at lower rates in the presence of 50 mM carbonate or phosphate (pH 9). The degree of the inhibition depends on the electron donor present in the solution. In the absence of any donor, carbonate suppresses almost completely lactate formation while, in the presence of 50 mM TRIS, the reduction rate is only decreased by about 30%. This effect can be rationalized by considering the known specific adsorption of anions at the TiO_2 surface, which occurs even at basic pH.[13,14] Through carbonate or phosphate binding at the surface of the particles, surface sites are blocked preventing the access of pyruvate and hole scavengers to the interface.

Pyruvate–TiO_2 interactions were further analyzed by measuring the adsorption of this anion on the semiconducting powder. The enzymatic determination of the amount of pyruvate left in supernatants of centrifuged TiO_2 suspensions showed a rather small binding (at pH 9, 7–16 μmol of pyruvate g^{-1} of TiO_2, which is the limit of detection of the technique is used) compared to the number of the surface hydroxyl sites. Furthermore, adsorption experiments done in the presence of TEOA, TRIS, or carbonate showed a suppression of pyruvate adsorption. These two observations support the idea that only weak specific interactions could possibly be involved in the interfacial electron transfer mechanism, at least at basic pH.

Another TiO_2 powder characterized by a higher specific surface area (Bayersol TiO_2, 145 m^2 g^{-1}) has also been tested in the photoassisted pyruvate reduction. The rate of lactate production is comparable to that obtained with P25 and the conversion efficiency obtained after the almost complete disappearance of pyruvate is similar for both powders. This indicates that under our experimental conditions the surface area is not the determining factor in the pyruvate reduction.

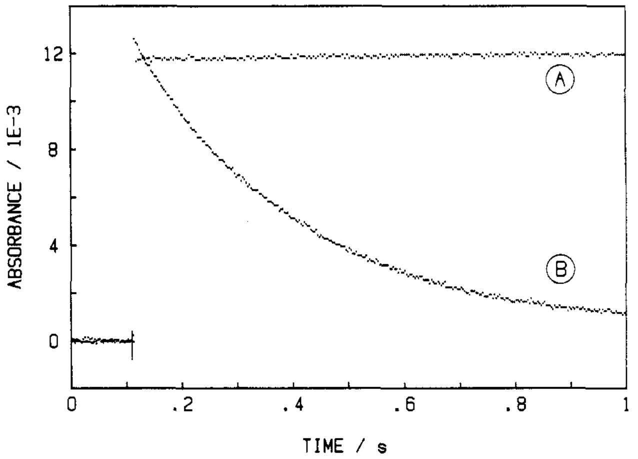

Figure 3. Time course of absorbance change (800 nm) after a laser flash (347 nm): (A) 0.5 g of colloidal TiO_2 L^{-1}, 25 mM TRIS, pH 9; (B) same conditions + 2.5 mM pyruvate.

Flash Photolysis. The use of colloidal semiconductors has already proved to be of great importance in the study of the dynamics of interfacial charge-transfer processes. The transparent nature of the sols obtained with ultrafine TiO_2 particles renders the application of laser flash photolysis and time-resolved adsorption spectroscopy feasible and thus allow for a direct determination of rate constants for the interfacial redox reactions.[15] The adsorption of photons of higher energy than the band gap of the semiconductor (3.2 eV for anatase, corresponding to λ = 388 nm) leads to the generation of electron–hole pairs. These charge carriers subsequently recombine or diffuse (in a few picosecond) to the surface of the colloidal particles (11 nm diameter) where they can undergo reactions with suitable scavengers. Valence band holes are either trapped by surface OH sites and form stable titanium peroxides, or they are removed by hole scavengers like TRIS molecules, allowing for conduction band electrons to remain in the particles and to undergo further interfacial electron-transfer reactions.

The absence of any noticeable visible light adsorption by both pyruvate and lactate precludes the study of redox processes through the direct observation of the concentration changes of these reactants. Conduction band electrons trapped in defects of the semiconducting particles display, however, a characteristic absorption band with a maximum around 780 nm at basic pH.[16] Recording the dynamics of optical density changes in this band will thus allow for the study of the kinetics of electron photoexcitation in the colloid and subsequent interfacial transfer to an acceptor such as pyruvate.

Preliminary studies of the stability of colloidal TiO_2 suspensions showed that in the absence of any protective agent the sols floculated even at low pyruvate concentration (1 mM) for all pH under 8.5. The experiments reported here have thus been done at pH 9 in colloidal suspensions containing 0.5 g L^{-1} of TiO_2 and 25 mM TRIS buffer, which is an efficient electron donor as shown by previous experiments on TiO_2 powders. In the absence of pyruvate, laser excitation induces a fast absorbance increase (observed at 800 nm in this study) which remains stable for more than 2 s, showing that well deoxygenated TiO_2 sols can be used even in slow electron-transfer reactions, provided that the intensity of the analyzing light is stable enough (Figure 3). When pyruvate is added, the initial absorbance increase is followed by a decay corresponding to first-order kinetics, as shown by the linear re-

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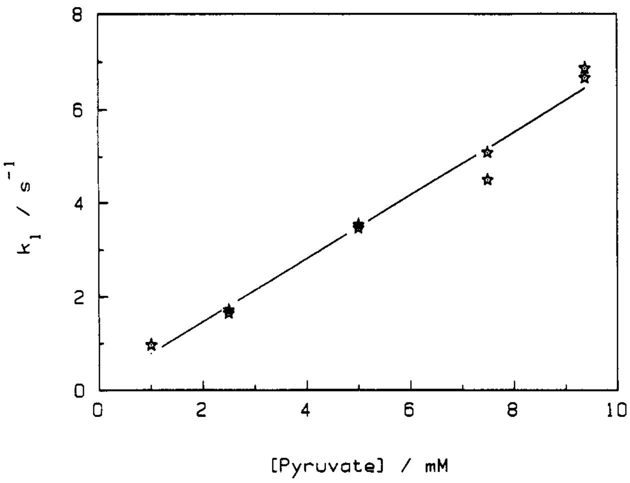

Figure 4. Plot of the observed pseudo-first-order rate constant vs. pyruvate concentration in 0.5 g L^{-1} colloidal TiO_2 and 25 mM TRIS, pH 9.

lationship obtained in a semilogarithmic plot.

Comparison between the initial photoexcited electron concentration observed in the TiO_2 sol just after the laser flash (about 30 μM) and the pyruvate concentration (1–10 mM) showed that interfacial charge transfer can well as described by pseudo-first-order kinetics. By varying the pyruvate concentration a second-order rate constant $k_2 = 680 \pm 50$ M^{-1} s^{-1} was derived from the linear relationship between the acceptor concentration and the observed pseudo-first-order rate constant (Figure 4). Interfacial electron transfer can be expressed by an heterogeneous rate constant k_{et} (cm s^{-1}) which is related to the measured bimolecular parameter via

$$k_{et} = 10^{-1}k_2/N_A(4\pi)r^2$$

where N_A is the Avogadro number and r is the radius of the colloidal particles in m.[17] For electron transfer is the presence of pyruvate a value of $k_{et} = 0.3 \times 10^{-6}$ cm s^{-1} has been determined.

Photoreduction of Other Compounds. Other photoassisted reduction reactions have been briefly tested to further investigate the mechanisms of electron transfer involved at the irradiated TiO_2 surfaces and to explore a little more the possible applications of this photosynthetic process. Reduction has been checked after 1 h of irradiation of a 0.5 g L^{-1} P25 TiO_2 suspensions containing 50 mM TEOA (pH 7) and 5 mM of the compound under study. Except for acetaldehyde, our investigation has been restricted to carboxylic acids which were analyzed by the experimental method already used for the pyruvate/lactate couple. The analytical conditions have, however, not been optimized in each case and the following results are thus only qualitative. Retention times (RT) indicated below have been measured by using 50 mM KH_2PO_4 (pH 3.4) as the mobile phase.

No evidence of any reduction have been obtained upon irradiation of samples containing carbonate, formate, or acetaldehyde. In the presence of glyoxylate ($HCOCOO^-$, RT = 4.34 min), three peaks appeared on the chromatogram, the highest one corresponding to glycolate $H_2COHCOO^-$, RT = 2.02 min). The surface area of this peak showed that about 2 mM of glycolate appeared after 1 h of irradiation. 2-Oxoglutarate ($^-OOCCH_2CH_2COCOO^-$, RT = 5.34 min) was also converted mainly to a product characterized by RT = 2.82 min, and a correlation between 2-oxoglutarate disappearance and the growning of the 2.82-min peak was observed. This last peak cannot, however, be assigned to the product of the carbonyl reduction of 2-oxoglutarate because no standard for the purified compound has been available. Interestingly, no change in the chromatograph has been observed when the analogue 3-oxoglutarate was added to the TiO_2 suspension, showing that the position of the carbonyl group relative to the carboxyl is important in the photoreduction process. Finally, we also observed the photoconversion of oxalacetate ($^-OOCCH_2COCOO^-$, RT = 3.02 min) to malate ($^-OOCCH_2HCOHCOO^-$, RT = 3.88 min) upon irradiation. The two peaks were, however, not sufficiently resolved to allow for a correct quantification of the two compounds.

Conclusion

Light-induced chemical transformations using semiconducting material for both photon harvesting and heterogeneous catalysis have been actively investigated recently for direct conversion of solar energy into chemical energy. The main objective in this domain remains the development of heterogeneous systems able to perform the efficient photoproduction of raw material by redox reactions such as the splitting of water, or the reduction of carbon dioxide and nitrogen. Another aspect of the possible use of semiconducting materials, particularly as finely divided particulate systems, consists in the utilization of their photon absorption and surface properties to perform photoredox organic synthesis. The reduction of pyruvate to lactate in irradiated TiO_2 suspensions represents a model reaction for such applications.

Although the mechanisms of this electron transfer has not been elucidated in detail, the observation that carbonyl groups adjacent to a carboxyl function can be catalytically reduced at the TiO_2 surface in the presence of different types of electron donors (alcohols, ternary amines, pyruvate) indicates that the reductive process is less complex than the chemistry involved in the oxidation of organic material by valence band holes.[2,18] Preliminary electrochemical experiments with TiO_2 electrodes have confirmed this finding.

The last comment concerns the energetics of the photoreduction. Although no evaluation of the quantum efficiency is available, indications on the conversion efficiency of the photoreduction can be obtained by comparing lactate formation with hydrogen evolution rates. Under the same conditions of irradiation, platinized TiO_2 produces 0.5–3 mmol of H_2 g^{-1} of TiO_2 h^{-1}, depending on the electron donor used, showing that the two-electron–two-proton reduction of pyruvate on the base TiO_2 surface is more efficient than the Pt-catalyzed H_2 production.

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Registry No. TiO_2, 13463-67-7; pyruvate, 127-17-3; lactate, 50-21-5.

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