Engineering *Acidithiobacillus ferrooxidans* Growth Media for Enhanced Electrochemical Processing

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DOI 10.1002/aic.14628

The chemolithoautotroph Acidithiobacillus ferrooxidans has been proposed as a potential electrofuel synthetic platform, and its growth medium is engineered to increase its conductivity and energy density, thereby improving viability of the process. The ion V^{3+} is used as an indirect electron supplier together with Fe^{2+} to grow A. ferrooxidans to increase the energy density of the medium, overcoming the Fe^{3+} solubility limit. A medium containing 10 mM Fe^{2+} with 60 mM V^{3+} was able to support cell growth to a final cell concentration very similar to medium of 70 mM Fe^{2+}. Integration of the biological process with an electrochemical reactor requires, for economical operation, a medium with high ionic conductivity. This is achieved by the addition of salt, and Mg^{2+} was found to be least toxic to the bacterium. A concentration of 500 mM Mg^{2+} is optimal considering constraints on bacterial growth and electrochemistry. © 2014 American Institute of Chemical Engineers *AIChE J*, 60: 4008–4013, 2014

Keywords: electrofuel, iron-oxidizing bacteria, redox mediator, vanadium

Introduction

Acidithiobacillus ferrooxidans is an obligate aerobic, acidophilic chemolithoautotroph which is able to extract energy from oxidation of reduced iron and sulfur compounds with an optimal pH range between 2.0 and 2.2.[1] *A. ferrooxidans* has been widely used in industrial biomining of sulfide ores and for the desulfurization of coal and sour gases.[2,3] Many of these applications exploit *A. ferrooxidans* as a catalyst to produce Fe^{3+}. The ferric ions can then oxidize sulfide compounds, and the reduced iron is then reoxidized by the bacterium. An example of the reaction used for flue desulfurization is[4]

$$SO_2+Fe_2(SO_4)_3+2H_2O=2FeSO_4+2H_2SO_4 \quad (1)$$

An example of the biomining reaction is[4]

$$CuS+Fe_2(SO_4)_3=CuSO_4+2FeSO_4+S \quad (2)$$

For these applications *A. ferrooxidans* grow using ferrous sulfate as the energy source and the biological reaction can be described as

$$4FeSO_4+2H_2SO_4+O_2=2Fe_2(SO_4)_3+2H_2O \quad (3)$$

Both processes require Fe^{3+}, and the solubility of ferric species is a strong function of pH. Recently, *A. ferrooxidans* has been proposed to have potential applications for production of chemicals or fuels[5] in a process termed "electrofuels."[6,7] In an electrofuels process, chemolithoautotrophic organisms utilize electricity either directly or indirectly as an energy source and carbon dioxide as carbon source.[8] To explore *A. ferrooxidans* as an organism for electrofuel production, we have genetically modified the organism to produce isobutyrate and heptadecane (Kernan et al., Submitted), and we have characterized the maintenance energy requirements and growth kinetics in continuous chemostat cultures.[9] The pH of the growth media was found to correlate with the maintenance energy coefficient. Although lower pH allows for higher Fe^{3+} solubility, it concomitantly requires a larger maintenance coefficient, which may translate into lower process efficiency.

For sustainable applications of the technology, it may be desirable to recycle the bioreactor effluent, which is comprised of oxidized iron species (Figure 1). One means of reusing the reactant is to electrochemically reduce the effluent. The iron is recycled and thus the only input into the overall process is water, oxygen, carbon dioxide, and electricity, but the electrical energy costs may be significant, depending on the electrode kinetics and the ohmic potential drops within the electrochemical cell. Furthermore, to minimize capital costs associated with electrochemical and biological reactors, high concentrations of iron (the energy source for *A. ferrooxidans*) are desired in the medium. The high concentrations allow for operation of the electrochemical reactor at high current densities (reaction rates per area), and should allow for a higher density of *A. ferrooxidans* cells in the bioreactor.

The power required for Fe^{3+} reduction can be expressed as: $P=(i \times A) \times V$, where i is the current density (mA/cm^2), A is the area of the electrode (cm^2), V is applied potential and $V=U_{\mathrm{thermo}}+\eta_{\mathrm{anode}}+|\eta_{\mathrm{cathode}}|+(i\times A)R_{\Omega}$, where U_{thermo} is the thermodynamic minimum potential, and η_{anode} and η_{cathode} are overpotentials associated with kinetic losses from the charge transfer reactions. The cell resistance R_{Ω} is inversely proportional to electrolyte conductivity. The conductivity in an electrochemical cell is a function of the ionic strength of the

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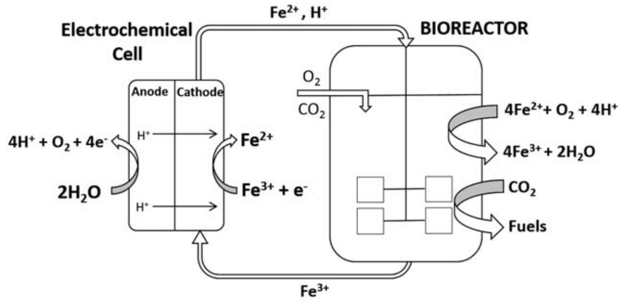

Figure 1. Schematic representation of electrofuel production system.

The right-hand side is a bioreactor, where genetically modified bacteria are cultivated, oxidizing Fe^{2+} to Fe^{3+} and producing a chemical or fuel. On the left-hand side, the electrochemical cell receives Fe^{3+} rich effluent from bioreactor and reduces Fe^{3+} to Fe^{2+} in the cathode. The Fe^{2+} rich media is then fed back to the bioreactor.

medium, and it is common in electrolyte design to include "supporting electrolyte" to increase conductivity.[10] As a means of minimizing the area A (and thus the capital investment in the electrochemical reactor), we must operate at a high current density i. To achieve this objective, it is necessary that the reactant concentration (Fe^{3+}) entering the reactor to be high. High iron concentrations also tend to reduce the requisite size of the bioreactor. In short, the integration of the electrochemical and biological reactors in an economically viable biosynthetic electrofuels platform requires the engineering of a medium that is high in ionic strength and iron concentration.

ATCC 2039 medium was used for cultivating *A. ferrooxidans*, which includes 72 mM Fe^{2+} and minimal amount of basal salts. This medium has low electrolyte conductivity and energy density. To increase the energy density, the solubility of the Fe^{3+} product must be addressed as Fe^{3+} solubility is significantly limited by pH. Theoretically, less than 10 mM Fe^{3+} is soluble at pH 2.2. This greatly limits the energy density in the system. Fe^{3+} precipitates can also foul electrodes and block tubes and valves of the system. The solubility of Fe^{3+} can be enhanced by lowering pH, but the biological efficiency declines. Previously, we showed that chelating agents, such as citrate, could be used to culture *A. ferrooxidans* cells with a high Fe^{2+} concentration at high pH values.[9] Introduction of citrate up to 70 mM into the culture medium successfully eliminated Fe^{3+} precipitates at the highest pH tested of 2.2. However, the inclusion of citrate to the culture media may reduce the electrochemical efficiency, increase cost, and encourage heterotrophic contamination. Thus, we chose to explore the inclusion of an additional redox couple with higher solubility than iron which can indirectly transfer reducing electrons to *A. ferrooxidans*.

Considering the natural habitat of *A. ferrooxidans*, the bacterium is inherently tolerant to the presence of heavy metals such as zinc, vanadium, and nickel, and we considered several metals as complementary redox mediators, to effectively increase the energy density of the media. For a soluble redox mediator to work, the redox couple must have a standard redox potential lower than that of the Fe^{3+}/Fe^{2+} couple (Eq. 4), so that reduced form is able to reduce Fe^{3+} back to Fe^{2+}. The reduced product Fe^{2+} can then continue to supply electrons to the bacteria. We looked specifically into Group 4 transition metals, which usually possess more than one oxidative state, and are readily accessible. The V^{4+}/V^{3+} couple, V^{4+}/V^{2+} couple, V^{3+}/V^{2+} couple, and Cr^{3+}/Cr^{2+} couple are examples (Eqs. 5, 6, 7, and 8), and these were investigated. However, V^{2+} ions are unstable in acidic condition and were found to undergo spontaneous oxidation in the media. Also, in acidic environment, Cr^{2+} can react spontaneously with protons to produce hydrogen gas, upsetting the pH balance, and rendering the media difficult to use in an industrial setting. This makes the V^{4+}/V^{3+} couple the most attractive choice. Furthermore, the solubility of vanadium ions is significantly larger than Fe^{3+} over a large pH range.

$$Fe^{3+} + e^- \iff Fe^{2+} \qquad E^0 = 0.77V \tag{4}$$

$$VO^{2+} + 2H^+ + e^- \iff V^{3+} + H_2O \qquad E^0 = 0.34V \tag{5}$$

$$VO^{2+} + 2H^+ + 2e^- \iff V^{2+} + H_2O \qquad E^0 = 0.60V \tag{6}$$

$$V^{3+} + e^- \iff V^{2+} \qquad E^0 = -0.26V \tag{7}$$

$$Cr^{3+} + e^- \iff Cr^{2+} \qquad E^0 = -0.41V \tag{8}$$

In this article, growth of *A. ferrooxidans* in a mixed Fe^{2+}/V^{3+} media was investigated. It was found that bacteria were able to grow in the mixed Fe^{2+}/V^{3+} media and this prevented ferric precipitation even at a pH of 2.2. The electrolyte conductivity was enhanced by addition of $MgSO_4$. These results indicate that use of V^{4+}/V^{3+} couple as soluble redox mediator and Mg^{2+} as electrolyte conductivity enhancer may facilitate development of an electrofuel biosynthetic platform using *A. ferrooxidans* cells.

Material and Methods

Microorganism and media

The *A. ferrooxidans* (ATCC 23270) bacterium used in this research was obtained from American Type Culture Collection (ATCC) and used throughout the study. The growth media contained 0.8 g/L $(NH_4)_2SO_4$, 2 g/L $MgSO_4 \times 7H_2O$, 0.4 g/L K_2HPO_4, 5 mL/L Wolfe's Mineral Solution, and 20 g/L (72 mM) of $FeSO_4 \times 7H_2O$ and was adjusted to pH 1.8. Exponentially growing bacteria were cultivated using 1-L batch cultures by weekly subculture at 30°C in an incubator shaker (Eppendorf, Hauppauge, NY) at 225 rpm. All chemicals were from Sigma Aldrich (St. Louis, MO) unless otherwise noted.

Biological batch experiment

Three groups of batch culture experiments were conducted to investigate the effect of vanadium on bacterial growth. In each experiment the same concentration of basal salts, that is, 0.8 g/L $(NH_4)_2SO_4$, 2 g/L $MgSO_4 \times 7H_2O$, 0.4 g/L K_2HPO_4, and 5 mL/L Wolfe's Mineral Solution was used and the concentrations of iron and vanadium were varied. An initial cell concentration equivalent to OD_{600} of 0.01 was used in each experiment. The pH was adjusted to 1.8 using sulfuric acid. Each experiment was conducted in 250-mL shake flasks using 100 mL as working volume. In the first group of experiments, growth of *A. ferrooxidans* on single metals was assessed. Cultures containing 70 mM of $FeSO_4$, $Fe_2(SO_4)_3$, $V_2(SO)_3$, or $VOSO_4$ were prepared and growth was compared to negative control cultures without a redox mediator. Cell growth was monitored by measuring cell density in each culture. In the second group of experiments,

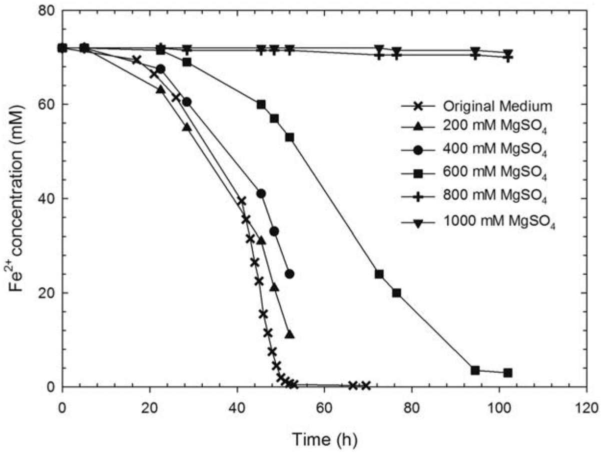

Figure 2. Biological depletion of Fe^{2+} as a function of time for different MgSO$_4$ concentrations.

bacteria were initially grown on 10 mM $FeSO_4$ in four different batch cultures. After the cells had consumed all the Fe^{2+}, 60 mM of $FeSO_4$, $Fe_2(SO_4)_3$, $V_2(SO)_3$, or $VOSO_4$ were added and cell densities were monitored. In the third group of experiments, bacteria were initially grown on 10 mM $FeSO_4$ in pH 2.2 batch cultures. 60 mM $FeSO_4$ or $V_2(SO)_3$ was added after cells had oxidized all of the Fe^{2+} and cell densities were monitored. The effect of Mg^{2+} on bacterial growth was also measured using batch cultures. Five 100 mL cultures at pH 1.8 containing 72 mM Fe^{2+} were supplemented with 200, 400, 600, 800, or 1000 mM $MgSO_4$ and bacterial growth was monitored by detecting depletion of Fe^{2+}.

Iron concentration and cell mass measurement

The Fe^{2+} concentrations in the cultures were measured by titration of 0.1 M cerium(IV) sulfate into mixtures of media samples (1 mL) and ferroin indicator (0.1 mL), and observing the color change.[11] Cell densities were measured in two ways, depending on whether precipitated salts or oxides were present. When precipitates were formed in the cultures, cells densities were measured using SYBR Green I (Invitrogen, Grand Island, NY) nucleic acid stain. When cultures were free from precipitates, an absorbance method was used. Both methods were described in a previously published article.[9]

Electrochemistry

Electrochemical kinetic experiments were performed on a Pt rotating disk electrode at 500 rpm with an IVIUM Multi-Channel Potentiostat. Analysis was done with linear sweeps at 5 mV/s over a range from 0.6 to −0.8 V vs. Ag/AgCl.

Results

High energy density and electrolyte conductivity in the growth media are desired to develop *A. ferrooxidans* as a biotechnology platform for chemical production. Several salts were explored ($MgSO_4$, Na_2SO_4, K_2SO_4, and $(NH_4)_2SO_4$) to determine their impact on bacterial growth (data not shown). $MgSO_4$ was found to be the least toxic to the cells. The highest concentration of Mg^{2+} that bacteria could tolerate in this study was 600 mM (Figure 2).

Preliminary experiments were conducted to determine if *A. ferrooxidans* could directly extract electrons from the oxidation of V^{3+} or V^{4+} (Figure 3). As they are both Period 4 elements, vanadium and iron have similar outer shell electron cloud distribution, and thus it could be possible for the cells to directly oxidize vanadium. Batch cultures containing 70 mM Fe^{2+}, 70 mM V^{3+}, 70 mM Fe^{3+}, or 70 mM V^{4+} with basal salt nutrients were cultivated. Cell growth was monitored as the change in cell density with time (Figure 3). Although cells grew on Fe^{2+} as expected, there was no growth in the V^{3+}, Fe^{3+}, or V^{4+} containing cultures.

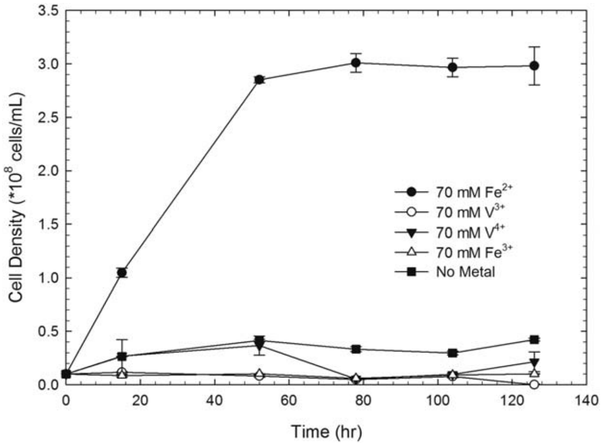

Figure 3. Change in cell density with time.

Growth curves are shown for cell culture at pH 1.8 using Fe^{2+}, Fe^{3+}, V^{3+}, and V^{4+}. Experiments were conducted in triplicated and reported in average. Error bars represent standard deviation ($n = 3$).

Experiments were conducted to test whether vanadium could serve as an indirect energy source to support cell growth. Cultures containing 10 mM Fe^{2+} were cultivated until all the iron was oxidized in each culture. Then, 60 mM Fe^{2+}, 60 mM V^{3+}, 60 mM Fe^{3+}, or 60 mM V^{4+} were added. Cell concentrations as a function of time were monitored (Figure 4). Results showed that cultures initially growing on 10 mM Fe^{2+} and then supplemented with 60 mM Fe^{2+} or 60 mM V^{3+} reached similar final cell concentration. As the cells cannot use V^{3+} directly, these results suggest cells in the V^{3+} supplemented cultures actually grew on the Fe^{2+} from Fe^{3+} that was reduced by V^{3+}. This conclusion

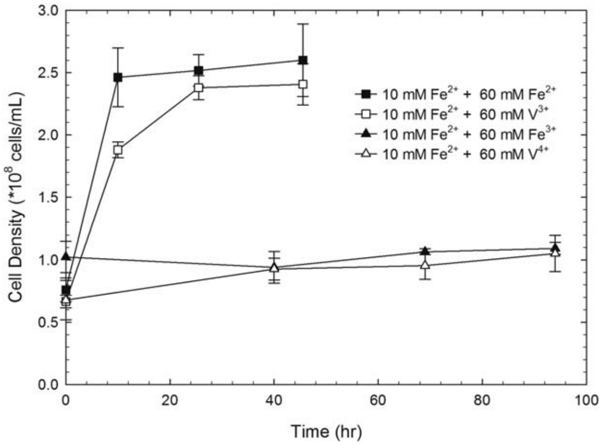

Figure 4. Change in cell density with time.

Growth curves are reported showing continuation of growth at pH 1.8 after consumption of 10 mM Fe^{2+} with addition of 60 mM Fe^{2+}, Fe^{3+}, V^{3+}, and V^{4+}. Experiments were conducted in triplicated and reported in average. Error bars represent standard deviation ($n = 3$).

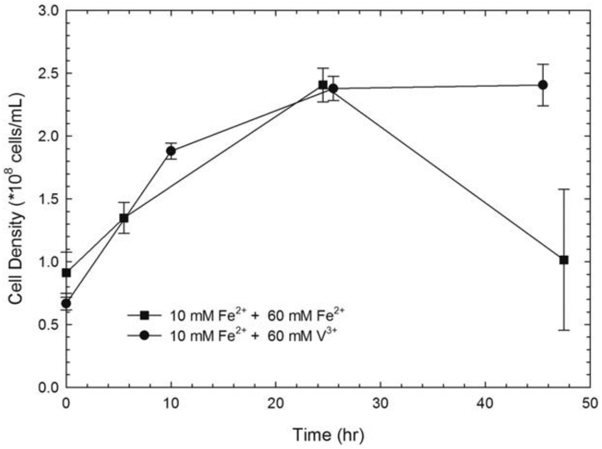

Figure 5. Change in cell density with time.

Growth curves are reported showing continuation of growth at pH 2.2 after consumption of 10 mM Fe^{2+} with addition of 60 mM Fe^{2+} and V^{3+}. Experiments were conducted in triplicated and reported in average. Error bars represent standard deviation (*n* = 3).

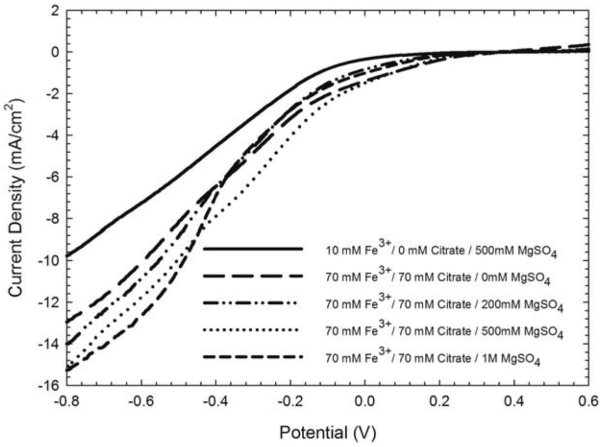

Figure 6. The current density as function of applied potential (relative to a Ag/AgCl reference electrode) at pH of 2.2.

Results are shown for different amounts $MgSO_4$. As a reference, results are also shown with and without chelator. Without citrate, the ferric concentration is 10 mM because higher concentrations lead to precipitation reactions.

is also supported by the standard redox potential differences between Fe^{3+}/Fe^{2+} and V^{4+}/V^{3+} (Eqs. 4 and 5). V^{3+} is able to reduce Fe^{3+} on a 1:1 ratio. From the perspective of electron availability to the cells, 10 mM of Fe^{2+} plus 60 mM of V^{3+} is equivalent to 70 mM of Fe^{2+}. Thus, the same final cell concentrations were obtained in the two cultures.

V^{4+} solubility is significantly higher than that of Fe^{3+}. This provides a significant potential advantage for the operation of a Fe^{2+}/V^{3+} system at elevated pH without precipitation formation. To demonstrate this effect, cultures initially growing on 10 mM Fe^{2+} at pH 2.2 were supplemented with 60 mM Fe^{2+} or 60 mM V^{3+} and incubated (Figure 5). Both cultures grew to a very similar cell density at 25 h. After that, the cell density decreased in the Fe^{2+} supplemented cultures while cell densities plateaued in V^{3+} supplemented group. This is likely due to the heavy precipitates that can form in ferric rich cultures at pH values where its solubility limit is exceeded.[12] Fe^{3+} precipitates are able to deposit onto the cell membrane, blocking diffusion of nutrients and gases. With a reduced oxygen supply, cells may not be able to neutralize protons and maintain their required intracellular pH gradient (Eq. 3). In the V^{3+} supplemented culture, the media was visibly free from precipitates; 1 mL culture samples were spun down by centrifugation, and no sediments were observed.

A goal of this work is to develop media compositions that support cellular growth and improves the electrochemical reduction of the iron in the media. In Figure 6, the first two entries show that for all the potentials tested, the current density increases with higher Fe^{3+} concentration. pH was set at 2.2, as this pH range leads to higher biological efficiency and citrate was added to prevent precipitation. It was found that current density further increased when $MgSO_4$ was added as a supporting electrolyte to improve electrolyte conductivity. At −0.8 V, the current density was found to be a maximum at 500 mM $MgSO_4$ and further increasing $MgSO_4$ to 1 M concentration did not increase the current density, as the ohmic potential losses were already negligible at these high ionic strengths. Nevertheless, at larger scales, the ohmic losses are more important, and additional $MgSO_4$ could improve electrochemical performance. Combining the present results with the Mg^{2+} bacterial growth compatibility studies, it is concluded that an additional 500 mM $MgSO_4$ to the culture media can benefit the entire system.

An alternative to introducing a chelating agent is to utilize the more soluble vanadium couple as an alternative energy source to both address Fe^{3+} insolubility and to increase the energy density of the media. Figure 7 shows polarization curves for three media compositions. Here, the pH was set at 1.8 to avoid precipitation when the high Fe^{3+} electrolyte is used. The 70 mM Fe^{3+} curve shows the largest current density at a given potential. The other two curves contain the same molar equivalents of reducible species in the solution, but show very small current densities. This suggests that the reduction of V^{4+} is kinetically slow. In short, the vanadium reaction only occurs at an appreciable rate at sufficiently

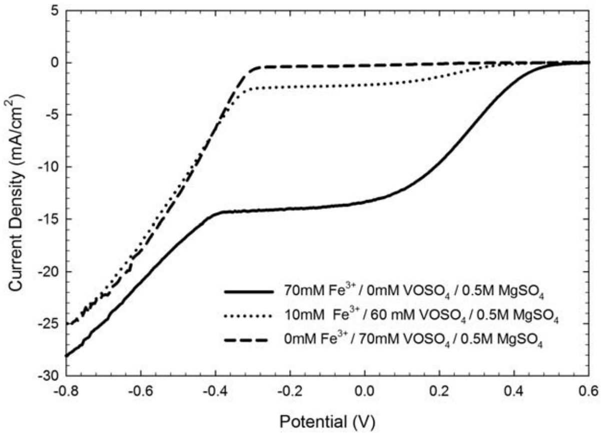

Figure 7. The current density as function of applied potential (relative to a Ag/AgCl reference electrode) at pH of 1.8.

Results are shown for the same molar concentrations of reducible ions (vanadium plus ferric ions). The dashed line shows that the kinetics of vanadium reduction is poor at this pH value.

negative potentials (less than ~ -0.4 V) where H_2 gas is coevolved. We have confirmed that indeed the vanadium can be reduced electrochemically in this regime based on the colorimetric changes from blue to green (corresponding to the solution that is predominantly V^{4+} to one that is V^{3+}) in the media. However, the evolution of H_2 represents a serious limitation as it results in a lower operational efficiency. Practical use of the vanadium couple may then require development of novel electrocatalysts.

Discussion

A. ferrooxidans cells are an attractive choice for the development of an electrofuels platform, as iron is readily electrochemically reducible. We have recently reported the genetic modification of *A. ferrooxidans* cells to produce isobutyric acid and heptadecane (Kernan et al., Submitted). Such successes are only a first step for the technology, as any process must be scalable and must achieve production rates per volume that allow for manageable capital costs. Development of a novel sustainable electrofuel production platform with iron circulating between an electrochemical cell and a bioreactor will require careful development of compatible media formulations. Both reactors benefit by increases in energy density of the medium. The electrochemical reactor, for example, can operate at higher current densities, and the bioreactor may be able to maintain higher cell densities.

In conventional biofuel processes, heterotrophic bacteria such as *E. coli* or yeast may be utilized, and they convert organic substrates into final products such as ethanol in a fermentation process. The organic substrates, for example, glucose, are usually fed in high concentrations at around 200 g/L (1.1 M) to yield a high final product concentration.[13] The high final product concentration leads to favorable process economics and facile separation. The energy density of the culture media is generally proportional to final product concentration. At a given productivity, higher product concentration reduces the size of the reactor, lowering the capital cost. In the electrofuel system, the *A. ferrooxidans* medium typically contained ~72 mM Fe^{2+} and thus the thermodynamically available energy from the oxidation of the irons (Eq. 3) is relatively small compared to what is available from glucose oxidation performed by heterotrophs.[1] The available energy (Gibbs free energy) from transferring 1 mole of electrons from Fe^{2+} to O_2 is approximately 34 kJ/mol Fe^{2+}, while in yeast fermentation, 1 mole of glucose yields about 120 kJ.[14] On a per liter basis, the glucose feed is ~130 kJ/L, and the iron feed is ~2.5 kJ/L at 72 mM, thus engineering the energy density of the media will be important.

The energy density of the media is proportional to Fe^{2+} concentration, but the soluble Fe^{3+} concentration is significantly limited by pH. Theoretically, at a pH of 2.2 less than 10 mM Fe^{3+} is soluble. Conversely, a high concentration of Fe^{2+} or Fe^{3+} may also impede cell growth due to substrate or product inhibition. Fe^{2+} concentration higher than 45 mM[15] and Fe^{3+} concentration higher than 36 mM[16] have been reported to inhibit cell growth. The use of vanadium as an indirect energy supplier is able to address the two problems. *A. ferrooxidans* has already been employed to extract vanadium from spent catalysts,[17] where sulfur species of high reducing power were exploited. Here, redox reactions between vanadium and iron ions were explored. We have shown that at a pH of 2.2, the culture growing in media of 10 mM Fe^{2+} supplemented with 60 mM V^{3+} achieved similar growth rate as the all iron culture (Figure 5). By using V^{3+} augmented with a small amount of Fe^{2+}, both the solubility limitation and ferric inhibition are resolved. At the same time, the mixed media contains the same energy density. This indicates that by using vanadium, it is possible to improve the energy density of the media while not causing precipitation and inhibition. The critical tolerance limit concentration of V^{4+} to the bacteria was reported to be 5 g/L, that is, 98 mM.[18] This roughly doubles the theoretical energy density of the Fe^{2+}/V^{3+} media, but it is still significantly lower than the glucose energy densities in heterotrophic media.

There is already established theory that redox potential of the medium, that is, Fe^{2+}/Fe^{3+} has a significant effect on cell growth.[1,19] At higher Fe^{2+}/Fe^{3+} ratio, cells have been reported to exhibit a larger iron oxidation rate.[19,20] In the Fe^{2+}/V^{3+} system described in this article, where reduction of Fe^{3+} by V^{3+} is always kinetically faster than biological Fe^{2+} oxidation, Fe^{2+}/Fe^{3+} ratio will be mostly high during growth due to constant turnover of Fe^{2+}, compared to the normal Fe^{2+} medium. The use of the Fe^{2+}/V^{3+} system hence could potentially increase iron oxidation rate of the cells, which is a crucial factor in the proposed electrofuel application.

An alternative is to employ a chelator like citrate to avoid precipitation of Fe^{3+} at higher pH. We have demonstrated that this is a viable approach, but this does not significantly improve energy densities beyond what we have achieved with vanadium media. Furthermore, both vanadium and citrate impact the energy requirements of the electrochemical reactor, and this must be considered in any analysis. In the industry, vanadium redox flow batteries (VRBs) have been extensively studied and implemented for large scale storage of energy from highly variable generation sources, such as wind and solar power.[21] In VRBs, the undesired H_2 evolution can be minimized by applying suitable catalysts, for example, Ru-dioxide, to increase the overvoltage of H_2.[22] A VRB utilizes the V^{2+}/V^{3+} and V^{4+} (VO^{2+})/V^{5+} (VO_2^+) couples at 1 mol/L in 2–3 mol/L H_2SO_4 and achieve high current density at reasonable potentials. In our system, the V^{3+}/V^{4+} (VO^{2+}) couple has its inherent disadvantage that it requires removing an oxygen atom from the vanadium atom in the reduction process, and this can be inefficient.[23,24] Thermodynamically, use of V^{2+}/V^{3+} could also reduce Fe^{3+}, but as discussed in Introduction, V^{2+} is very unstable in acidic environment. Increasing vanadium concentration in the medium will improve current density but biological tolerance is a problem. Therefore, the vanadium-media approach will require further development of electrocatalysts. Conceptually, an electrofuel system has similarities to VRBs, in terms of the storage of electrical energy, except the electrical energy is transformed into a chemical product.

Efficient operation of the electrochemical reactor also requires media with good ionic conductivity, and we found that cells could tolerate up to 600 mM $MgSO_4$. Other ions we explored, such as Na^+, K^+, and NH_4^+, have a large inhibitory effect on the cell growth. A previous report has indicated that divalent cations are less toxic to the cells as compared to monovalent cations.[25] Also, considering that magnesium serves as an essential elemental for the ferrous oxidation activity,[26] the ready adaption to high magnesium ions is expected. Nevertheless, the additional salts required by the electrochemical reactor could impact cell growth kinetics, possibly lowering achievable cell densities in a bioreactor. Thus, considering the lower energy densities available in the inorganic media, there is a need for novel designs that will permit achievement of large production rates. Addition of $MgSO_4$ to the media increases current density at a given applied potential (Figure 7) through reductions in the ohmic drop. The effect of additional $MgSO_4$ plateaued at 500 mM.

Conclusion

A. ferrooxidans have been widely used in biomining of crude copper ores and has been suggested for use in a novel electrofuel biosynthetic platform. In this study, engineering of *A. ferrooxidans* media to enable this application was explored. The soluble V^{3+}/V^{4+} redox coupled was shown to be compatible with the cells and improved the energy density while addressing solubility issues. The addition of Mg^{2+} also enhances conductivity. But adoption of the approach with the vanadium couple will require more intensive development of the electrochemical reactor.

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Manuscript received July 23, 2014, and revision received Aug. 28, 2014.