Active fluid with *Acidithiobacillus ferrooxidans*: correlations between swimming and the oxidation route

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Abstract

To explore engineering platforms towards ‘active bacterial baths’, we grow and characterize native and commercial strains of *Acidithiobacillus ferrooxidans* to promote swimming locomotion. Three different energy sources were used, namely elemental sulfur, ferrous sulfate, and pyrite. The characteristics of the culture, such as pH, Eh, and the concentration of cells and ions, are monitored to seek correlations between the oxidation route and the transport mechanism. We found that only elemental sulfur induces swimming mobility in the commercial DSMZ – 24,419 strain, while ferrous sulfate and the sulfide mineral, pyrite, did not activate swimming on any strain. The bacterial mean squared displacement and the mean velocity are measured to provide a quantitative description of the bacterial mobility. We found that, even if the *A. ferrooxidans* strain is grown in a sulfur-rich environment, it preferentially oxidizes iron when an iron-based material is included in the media. Similar to other species, once the culture pH decreases below 1.2, the active locomotion is inhibited. The engineering control and activation of swimming in bacterial cultures offer fertile grounds towards applications of active suspensions such as energy-efficient bioleaching, mixing, drug delivery, and bio-sensing.

Keywords Active fluid · Bacterial mobility · Mean squared displacement · *Acidithiobacillus ferrooxidans*

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1 Introduction

Biological matter relies on active processes at the molecular scale to drive and direct biological functions in a perpetually changing far-from-equilibrium thermodynamic state. Homeostasis in living things ensures that the chemical arrangements are maintained while atoms and molecules are exchanged constantly. Motors at the molecular scale are an important mechanism for homeostasis and they are present in almost every biological function, such as cell division, DNA propulsion, and molecular locomotion. Some of the biological entities that are driven by molecular motors exhibit large-scale collective behavior at the fluid state, resulting in what is commonly known as ‘active fluids’ [1–3]. Among the challenges imposed by our desire to mimic biological matter in technological applications, is the engineering ability to emulate molecular motors artificially, motivating efforts to generate the fundamental basis and the understanding of active fluids [4–15].

In particular, bacterial swimming and the resulting fluctuating hydrodynamic field have received special attention by biologists, physicists, and engineers because they have potential uses in microfluidic mixing, active drug delivery, bio-sensors, bio-inspired micro devices, bio-remediation, and bioleaching [16–23]. During the last couple of decades, several experimental setups have been developed to study these systems, where image analysis is used to provide information about bacterial trajectories, mean velocity, and the distinction between diffusive and swimming bacteria. From a theoretical point of view, coarse-grain bacterial models and simulations have served as a valid tool to study the complex behavior of active suspensions; for instance, they guided researchers in the understanding of the hydrodynamically induced collective motion and suspension stability [7, 13, 14, 24, 25]. In fact, some thermodynamic approaches have been proposed to understand active matter from a fundamental point of view [4, 26].

From a technological point of view, it is vital to understand swimming modes to design, optimize, and control bacterial motility. For example, a bio-reactor design where external mixing is not required may be energetically or technologically preferable. The use of bacterial reactors in mining, element bio-leaching, mud bio-remediation, or CO₂ absorption are sometimes discharged due to energy requirements and low efficiency. Driving bacterial swimming and controlling the motility mechanism may generate novel routes for the optimum design of robust bio-technological processes [27]. For instance, microorganisms have been used to bio-leach and bio-remediate solutions to achieve mineral separation. Gold extraction from sulfide ores (pyrite (FeS₂), chalcopyrite (CuFeS₂), arsenopyrite (FeAsS)), carbon desulfurization, phosphorus, copper, zinc and nickel extraction, kaolinite bleaching, and cyanide treatment [21, 22, 28–33], are examples of such biotechnologies.

Some bacteria swim by the rotation of flagella, which are distributed through the cell body. Swimming bacteria obtain nutrients over length scales much bigger than their size, whereas the driven hydrodynamic field induces bio-convection. Typically, swimming is characterized by linear displacements (run) interrupted by random reorientations (tumbling) [34–36], where the bacteria use chemical sensors, magnetic dipoles, and their adaptation capacity to determine the swimming mode. If flagella rotate in the same direction, they interact hydrodynamically, forming a collapsed form that exerts a uniform swimming force [37]. Rotating flagella in different directions, or rates, results in unequal hydrodynamic forces that generate a random rotation, or tumbling [38]. On the other hand, diffusion (Brownian motion driven) is a passive means of mobility, which is inefficient at long length scales. Many bacterial species have adaptation capabilities that vary according to the medium in which they are cultured; changing the mobility mechanism that results in the least amount of energy used. Therefore, if bacteria are cultured in

rich and well-mixed mediums, they inhibit genes that create flagellar proteins [30, 39–41]. On the other hand, sedimented or non-mixed media may force flagellar motion to be active. Recall that bacterial biology is enriched by physical and chemical mechanisms, which ensure survival with minimum energy consumption. When a particular species contains the genes to generate flagella or pili, to be able to sustain active locomotion, the ability to swim depends on factors like domestication, phenotype variation, or epigenetics of expression factors [40]. The flagellated bacteria *E. coli*, *B. subtilis*, *P. aeruginosa*, *V. alginolyticus*, and *P. mirabilis*, as well as the single-cell green alga *C. reinhardtii*, have been studied extensively [34, 42–48].

Acidithiobacillus ferrooxidans have been used widely in bio-leaching, mineral extraction, and mining applications worldwide [22, 32, 46–52]. There is some level of discrepancy and discussion regarding its capacity to form flagella and undergo swimming. However, Ohmura and coworkers [53] observed swimming and flagella when *A. ferrooxidans* ATCC-23270 were grown in a medium with elemental sulfur. This strain showed different motilities, depending on type IV pili, undergoing swarming and pyrite adhesion [54, 55]. However, Jerez [56] and Valdés et al. [57] argued that this strain lacks genes to form flagella. Conversely, strains SP5/1 and HV2/2, during pyrite oxidation [58] and *Thiobacillus ferrooxidans* (TFI-1, TFI-4, TFI-6, TFI-7, TFI-9, TFI-10 and TFI-13) isolated from a uranium mine in Canada [59], showed flagella and swimming motility. Motivated by these discrepancies and by the desire to design an energy-efficient bio-leaching process, we cultured several *A. ferrooxidans* strains in the presence of different energy sources to study bacterial mobility. The aim of this study is to be able to control the transport mechanism and to understand the effects of swimming in bio-oxidation and bacterial adaptation.

We start this paper with a description of the materials and methods, where two experimental stages, growing and swimming, are described. Culture characteristics and bacterial mobility are measured to identify the bacterial transport mechanism and the changes in bio-oxidation. Results are then presented, followed by the corresponding discussion; we then finish the manuscript with a summary of the most relevant conclusions.

2 Materials and methods

Bacterial strains Three *Acidithiobacillus ferrooxidans* strains are used: an isolated wild type, called Nativa-16 (N-16) and two commercial strains, ATCC-23270 (ATCC) and DSMZ-24419 (DSMZ). N-16 was extracted from the ‘Laboratorio de Biomineralogía y Biohidrometalurgia’s’ (LBB) bacterial strain collection, at the Universidad Nacional de Colombia. The LBB is supported and allowed to collect bacterial strains under Resolution 1647 (2011) from the ‘Autoridad Nacional de Licencias Ambientales’ (ANLA); and to access genetic resources according to Resolution 1285 (2013) from the ‘Ministerio de Medio Ambiente y Desarrollo Sostenible’ (Colombia). Commercial strains ATCC and DSMZ were purchased from the American Type Culture Collection [60] and the Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH [58], respectively.

A. ferrooxidans are Gram-negative bacteria, acidophiles and chemo-lithoautotrophic. They have the ability to grow in extreme environments and their energy source is obtained through the oxidation of reduced compounds, like Fe^{2+} ions, elemental sulfur (S^0), thiosulfate ($\text{S}_2\text{O}_3^{2-}$), and tetrathionate ($\text{S}_4\text{O}_6^{2-}$), or through the oxidation of ores like pyrite (FeS_2) [61], arsenopyrite (FeAsS), and chalcopyrite (CuFeS_2). They are able to select between direct or indirect leaching mechanisms [32, 50]. In the presence of a ferrous ion, *A. ferrooxidans* undergo a set of

chemical reactions that may be summarized into an initial transformation of the ion [46, 52, 61, 62]:

followed by the hydrolysis of the ferric ion, which precipitates to form ferrihydrites:

where ΔG^0 denotes the standard formation Gibbs free energy for each reaction [63–67].

On the other hand, in the presence of elemental sulfur, *A. ferrooxidans* oxidize it to produce sulfuric acid [66]:

Including an ore (like pyrite) in the medium forces *A. ferrooxidans* to oxidize Fe^{2+} to Fe^{3+} and to undergo a cycle that allows energy extraction following a direct process [46]:

or an indirect mechanism:

As mentioned before, there are discrepancies regarding the capacity of *A. ferrooxidans* to undergo active locomotion and whether diverse phenotypes can be expressed according to different energy sources. According to Silverman and Lundgren [60], *A. ferrooxidans* prefer Fe^{2+} ions over S^0 when cultured with both. Brock and Gustafson [68] and Suzuki and collaborators [48] observed variants during oxidation of Fe^{2+} ions and S^0 due to phenotype differences in aerobic and anaerobic conditions. Under electronic microscopy, *A. ferrooxidans* strains ATCC 19859, ATCC 23270, and ATCC 33020 did not exhibit flagella or pili [69] when grown in solid media with thiosulfate and iron. However, the authors observed variations on phenotypes associated with culture morphology and the oxidation of Fe^{2+} ions. Ohmura et al. [53] reported the presence of flagella and active mobility of the strain *A. ferrooxidans* ATCC 23270, once S^0 is used as an energy source. In addition, they detected proteins, associated with flagella adhesion to sulfur. Previous gene expression studies had raised questions about the genetic variability between multiple *A. ferrooxidans* strains. They revealed the importance of epigenetic and adaptability factors, like the extracellular protein expression for cell adhesion, the presence of pili type IV and the modulation of operons and transcription factors when cells are grown or are transferred from a media with Fe^{2+} to one with S^0 [54, 55]. Valdés, Pedroso and coworkers [57, 70] concluded from sequencing data that genes that encode flagella were not identified in the genome of strain ATCC 232070. Their results contradict those of Li and coworkers [54, 55] where they detected the presence of pili type IV and flagella on the same strain. It is important to highlight that the genetic variation among strains is an important factor

for the mobility mechanism. For instance, isolated DSMZ 24419 strains were shown to be flagellated in the presence of pyrite; the cells were able to adhere to the mineral with the pili [58, 59]. Therefore, we believe that presence of active mobility in *A. ferrooxidans* could be a consequence of phenotype expressions according to the energy source and the genetic variability between strains.

Growth – (S-I) In the first stage, the three strains were cultured with different energy sources in order to detect the combination that exhibits swimming mobility. Strains were grown at 30 °C in an effective volume of 100 ml with 10% (v/v) inoculum under no agitation in an Erlenmeyer flask of 200 ml. A 9 K ([60]) medium was used with basal salt concentrations (g/l) of $3(\text{NH}_4)_2\text{SO}_4$, $1/2\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $1/2\text{K}_2\text{HPO}_4$, 0.1KCl and 0.01 $\text{Ca}(\text{NO}_3)_2$. The pH was adjusted to 2 with H_2SO_4 .

Energy sources were 2.25 g of S^0 (elemental sulfur) and 10% (v/v) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (44 g/l Fe^{2+}) (ferrous sulfate) with and without 1.5 cm FeS_2 (pyrite) cubes. The later form was chosen to force the bacteria to move towards the mineral. The bacterial suspensions were grown for 16 to 20 days. Image analysis and measurements of pH and Eh were used to follow the culture dynamics.

Swimming bacteria – (S-II) The strain and conditions that drive the swimming motion were selected for the bio-oxidation experiments. The bacteria were grown further at 30 °C in an effective volume of 100 ml with 10% (v/v) inoculum in an Erlenmeyer flask of 200 ml. The 9 K medium was adjusted to pH = 1.8 with H_2SO_4 .

Energy sources were 2.25 g of S^0 , 10% (v/v) $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (44 g/l) and 1.25 g of S^0 with 5% (v/v) $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$. The three cultures were agitated in an orbital shaker at 180 rpm and kept for 20 days. Image analysis and measurements of pH, Eh, and Fe^{2+} , SO_4^{2-} and cell concentrations were made to follow the culture dynamics.

Cell-tracking analysis Cell tracking was performed by extracting 50 μl of cell cultures in a slide hemocytometer with 0.1 mm depth and 0.0025 m^2 area. The distance between the hemocytometer to the slide was 100–110 μm . A Nikon Alphaphot 2 microscope, with 40 $\times$ phase-contrast objective and equipped with a Motic cam 580 5MP video camera, was used. Video analysis for cell tracking was performed with ImageJ software [71]. An image filter was not used to avoid cell/noise confusion. Swimming cells move out of focus in long runs; therefore, 10s videos were used for velocity measurements. Tracking movies were typically of 200 frames of 0.058 s; the taking scale was 150 pixels/50 μm . During growth (S-I), tracking was done in specific cultures (as will be discussed later), whereas, for the swimming bacteria (S-II), it was carried out daily.

Mobility is characterized by measuring the mean squared displacement (MSD) of the cells [20, 72–75]. A direct and simple quantitative method to characterize bacterial mobility is through the power law exponent, n , of the time during the evolution of the mean squared displacement: $\langle \Delta x^2 \rangle \sim t^n$ [73]. The MSD behavior in time dictates whether cells are diffusing, swimming, or moving in intermediate regimes. The evolution of the MSD follows a Green–Kubo relation for the mobility tensor and allows the direct calculation of transport coefficients. The MSD correlates to time through $\langle (\mathbf{x}_{cm}(t) - \mathbf{x}_{cm}(0))^2 \rangle = 2dMt^n$, where $\mathbf{x}_{cm}$ is the cell center of mass, d is the dimensionality, M is the mobility coefficient, the power law exponent n determines the mobility regime and the brackets denote ensemble average over all cells. The characteristic length and time scales in bacterial suspensions determine the inertial particle time

around 10^{-9} to 10^{-12} s, i.e., the Reynolds number is practically zero and the motion is inertial-less. Consequently, an active suspension behaves ballistically with $n=2$ at short times, whereas a diffusive suspension undergoes a random walk driven by Brownian motion, the mobility coefficient is reduced to the thermal diffusion coefficient and $n=1$. This diffusion coefficient can be estimated from the Stokes–Einstein relationship by $D \sim k_B T / 6\pi\mu R$, where k_B is the Boltzmann constant, T the absolute temperature, μ the fluid viscosity, and R the hydrodynamic radius of the particles. On the other hand, the active (ballistic) suspension becomes diffusive (with $n=1$) at long times due to the large coherent and collective motions driven by the hydrodynamic interactions between the particles [9–16]. This diffusive regime is no longer due to Brownian motion, the coefficient cannot be estimated from Stokes–Einstein and it undergoes a random walk over length scales that are much larger than the particle characteristic size. We adopted the terms ‘ballistic’ or ‘active’ for the swimming suspensions that at short times $n=2$, whereas ‘diffusive’ to the suspensions driven by Brownian motion ($n=1$ at short times).

Analytical procedures Cell concentration, pH, and Eh were measured every 2 days during growth (S-I) and every day, from day 5, for the swimming bacteria (S-II). During S-II, Fe^{2+} and SO_4^{2-} are followed to relate their concentration with swimming behavior and bacterial concentration. Ferrous iron concentrations were measured with the 3500 FeB method, while sulfate is done following APHA 4500 [76]. A Genesys 10-UV spectrophotometer, Thermo Scientific®, was used to measure the concentrations of the energy sources and oxidation products. The pH measurements were performed in an HQ 40d-pH-meter (HACH Company®) with a KCl (PHC301) electrode. A Handylab potentiometer (SCHOTT Company®) with an Ag/AgCl (Pt-6880) electrode was used for the Eh. The pH meter and potentiometer were calibrated with a low pH buffer. Cell count was done directly on the slide hemocytometer.

3 Results and discussion

Growth – Stage I During growth, the cultures with different energy sources were followed and characterized in order to identify the combination that undergoes a ballistic locomotion at short times (active). Table 1 summarizes the type of mobility (swimming or non-swimming) observed for the three strains with four different combinations of energy sources. Strains N-16 and ATCC did not undergo swimming, whereas the DSMZ strain exhibits active mobility (swimming) when cultured in elemental sulfur or in elemental sulfur plus pyrite. These observations are consistent with previous works [53, 58] that reported mobility of DSMZ strains in sulfur-rich environments. One may conclude that a sulfur-containing mineral like pyrite may induce flagellar mobility as well; however, as shown in Table 1, swimming was not

Table 1 Cell mobility during growth with different sources energy for three strains: (–) non-swimming; (+) swimming/active

Energy source → Strain	$\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (SI-A)	$\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ and pyrite (SI-B)	S^0 (SI-C)	S^0 and pyrite (SI-D)
N-16	–	–	–	–
ATCC 23270	–	–	–	–
DSMZ 24419	–	–	+	+

Fig. 1 Typical trajectories for Brownian bacteria during stage I: **a** DSMZ 24419 in $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$; **b** N-16 in $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$; **c** N-16 in S^0 ; **d** ATCC 23270 in S^0 . The trajectories are composed of 200 frames every 0.058 s; the colors correspond to different cells. For illustrative purposes, only a few cell trajectories are drawn

observed in cultures with ferrous sulfate and pyrite. Therefore, only elemental sulfur was responsible for the flagellar mobility in DSMZ. This conclusion may be explained by gene-expression differences [77–79], because for elemental sulfur, there is evidence of protein expression that enhances adhesion to the mineral particles [53, 77] and activated genes that develop flagella.

Fig. 2 Typical trajectories for the swimming DSMZ strain during stage I: **a** strain with S^0 ; **b** strain with S^0 and pyrite. The trajectories are composed of 200 frames every 0.058 s; the colors correspond to different cells. For illustrative purposes, only a few cell trajectories are drawn

Fig. 3 Dynamics of the bacterial culture during growth - stage-I: **a** pH; **b** Eh; and **c** cell concentration [C]. These non-swimming strains were grown with ferrous sulfate

Typical time sequences for non-swimming and swimming bacterial suspensions are shown in Figs. 1 and 2, respectively. The trajectories are recorded over 11.6 s at 40 $\times$ augmentation, with 200 frames each of 0.058 s. Brownian motion is observed for the non-swimming bacteria, where the bacteria undergoes a random walk over distances that

Fig. 4 Dynamics of the bacterial culture during growth - stage-I: **a** pH; and **b** Eh. These non-swimming strains were grown with ferrous sulfate and pyrite

are greater than its size. Swimming bacteria follows a ballistic behavior at short-time scales, as indicated by the long 'straight' trajectories. In Fig. 3, we summarize the evolution of the pH, Eh, and the cell concentration $[C]$, for the non-swimming ATCC, N-16, and DSMZ strains. These cultures were grown in ferrous sulfate and the results in the figure are representative of the behavior of other non-swimming cultures without pyrite. During the first 6 days, a pH increase is observed, due to water formation and after day 6 the pH shows a monotonic decaying trend. Correlated with the initial pH is the increase in the Eh, related to the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio [46, 51] and the cell concentration. Notice that the kinetics and cell growth shown in Fig. 3 are similar between strains, which may be an indication that the strains belong to the same species. When the strains are cultured with pyrite, a set of additional oxidation kinetics are introduced, Eqs. (4) and (5), thereby resulting in faster pH and Eh responses, as shown in Fig. 4. There is a stronger increase in the initial pH level and a corresponding increase in Eh (and cell concentration, not shown). The pyrite oxidation mechanism accelerates growth and kinetics four days when compared to the kinetics without mineral. The use of pyrite as an alternative energy source provides means for culture engineering, offering alternatives to accelerate growth and increase process efficiency.

Fig. 5 Dynamics of the swimming DSMZ bacterial culture during growth - stage-I: **a** pH; and **b** cell concentration [C]. The elemental sulfur and the elemental sulfur plus pyrite drove swimming/active locomotion

The pH and cell concentration evolution for the swimming DSMZ strain grown in elemental sulfur and elemental sulfur plus pyrite are shown in Fig. 5. We are demarking, with dotted lines, the day where swimming mobility is observed: the 8th day for S⁰ and pyrite and the 12th day for S⁰. Even though the presence of flagella in the DSMZ strain was observed in the past in presence of pyrite [53], we only observed swimming mobility with elemental sulfur. Recall that in our experiments, DSMZ with ferrous sulfate and pyrite did not exhibit swimming (Table 1). As shown in Fig. 5, the inclusion of sulfur changes the oxidation kinetics and the culture behavior: there is a weak pH increase during the first 2 days, due to poor bacterial adaptation, with a uniform decrease during the remaining days. The final pH level, around 1.65, is the lowest value observed in these experiments. The cell concentration and Eh increase linearly with time.

Swimming DSMZ strain - stage II As described in the Materials and Methods section, after the DSMZ bacteria, using elemental sulfur as energy source (swimming mobility) was grown for 16 days, it was removed and cultured for an additional 20 days with three new energy sources, namely elemental sulfur S⁰, ferrous sulfate Fe₂SO₄·7H₂O, and S⁰ plus Fe₂SO₄·7H₂O. The idea was to correlate swimming and culture characteristics.

Fig. 6 Evolution of the trajectories for the swimming DSMZ strain during stage II. During this stage, the culture contains ferrous sulfate and elemental sulfur. Each trajectory is composed of 200 frames every 0.058 s; the colors correspond to different cells. For illustrative purposes, only a few cell trajectories are drawn

The swimming motion in these cultures is observed from day 2 to day 16, as shown in Fig. 6, where typical bacterial trajectories are illustrated for the culture with ferrous sulfate and elemental sulfur. After the 16th day, swimming was no longer observed in the cultures. Control experiments were carried out up to 45 days after the measurements to confirm that the bacterial culture was still alive. The time evolution of the culture pH and the cell concentration are summarized in Fig. 7. Our results confirm that the evolution of the cultures with S^0 and $Fe_2SO_4 \cdot 7H_2O$ undergo different chemical paths, demonstrated by the pH behavior. The culture with both energy sources follows an intermediate path, with a similar trend to the culture with only $Fe_2SO_4 \cdot 7H_2O$. These results reaffirm that *A. ferrooxidans*, even if grown with elemental sulfur, always prefer to follow the iron oxidation route. Notice that during the first 2 days, an initial ‘shock’ is observed and the pH is almost constant; once the bacteria realizes that there is iron in the medium, they activate this oxidation metabolism [52, 80]. The days where swimming was observed are marked between the dotted lines in Fig. 7: day 2 to 16. We found that once the pH level is below 1.2, swimming is inhibited [52]. In addition, the decrease in mobility evidences the main role of iron in the mobility. Cell concentrations are one order of

Fig. 7 DSMZ suspension behavior during swimming: **a** pH and **b** cell concentration $[C]$

magnitude higher than those during the initial growth in S-I; the increase is attributed to the fact that in the presence of Fe ions, *A. ferrooxidans* can oxidize simultaneously from other energy sources, like S^0 [79, 81].

To confirm the bacterial accommodation to the iron oxidation route, the concentrations of ferrous ion and sulfates were measured during evolution and are shown in Fig. 8. We included the active strains that were cultured with $Fe_2SO_4 \cdot 7H_2O$ as the unique or alternative energy source. For both cultures, the sudden decrease of the Fe^{2+} concentration proves that the bacteria even grown in a sulfur-rich environment undergo the iron oxidation route. In addition, when $Fe_2SO_4 \cdot 7H_2O$ is the only energy source, the sulfate concentration (SO_4^{2-}) remains constant; these sulfates are the final products of the initial growing stage. On the other hand, for the culture with $Fe_2SO_4 \cdot 7H_2O$ and S^0 , the ferrous ions are also decreased at a fast rate during the initial days, while the sulfates are constant up to the 11th day. Once the ferrous ions are consumed, the sulfate concentration increases, thereby indicating the activation of the sulfur oxidation mechanism. Notice that day 11 demarks the lowest value in the pH level, and the culture that includes elemental sulfur shows an increase in sulfates up to 650 g/l. Correlating the

Fig. 8 Dynamics of the Fe^{2+} and SO_4^{2-} ions during swimming of DSMZ bacterial suspensions

results in Fig. 8 with the activation of the swimming locomotion suggests that this type of mobility starts once the $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ is almost consumed. This may suggest that the unique presence of the elemental sulfur is central to the appearance of swimming cells. Our results are similar to previous works [79], where a similar trend in Fe^{2+} was observed changing the culture media from S^0 to $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$. We found it possible to conclude that, according to our results, a low pH inhibits the swimming locomotion. However, it is evident that the Fe^{2+} concentration is also responsible for observed differences in the mobilities.

The regime of mobility was quantitatively characterized by the behavior of the bacteria mean squared displacement (MSD). In Fig. 9, we include a representative measurement of the MSD as a function of time for a DSMZ culture with $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ and S^0 as energy sources. The MSD was tracked every day; the figure includes measurements for days 5 and 16, where a swimming/ballistic movement with $n=2$ and Brownian diffusive behavior with $n=1$, are observed. However, it is necessary to remember that these measurements were taken during few particle swimming or diffusive times; therefore, we do not capture the hydrodynamic driven diffusion of the active cells. Two typical trajectories to highlight the characteristic length scales of the motion are

Fig. 9 Mean squared displacement (MSD) as a function of time for DSMZ bacterial suspension during days 5 and 16. The ballistic (active) behavior at day 5 with $n = 2$ and the diffusive (Brownian) mobility at day 16 with $n = 1$ are shown. Typical trajectories of the active and Brownian suspensions are included to highlight the length scale of the bacterial transport

included in the figure for completeness. We found that the bacteria with $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ exhibits a maximum mobility in the ballistic regime of $10 \mu\text{m}^2/\text{s}^2$ at day 9 and averaged values between $8.1 \mu\text{m}^2/\text{s}^2$ and $11.9 \mu\text{m}^2/\text{s}^2$. These coefficients quantify the short-time ballistic/active transport of the bacteria during those days. On the other hand, for the Brownian suspension ($n = 1$), the averaged diffusion coefficient is $0.5 \mu\text{m}^2/\text{s}$, which corresponds to a particle with a hydrodynamic radius between 0.5 and $1 \mu\text{m}$ depending

Fig. 10 Mean velocity of the cells as a function of time for DSMZ bacterial suspension

on the fluid viscosity. This value is lower when compared with reported diffusion coefficients of *E. coli* of about $400 \mu\text{m}^2/\text{s}$ or the $101 \mu\text{m}^2/\text{s}$ of *V. alginolyticus* [34, 35, 44, 72–74, 82]. The mean velocity of the bacteria, obtained from video analysis, is shown in Fig. 10. The average velocity reaches a maximum during the first days of the swimming mobility and decreases monotonically in time. This observation is correlated with the low pH values and the decrease in the concentration of the ferrous ion. Cultures with $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ and S^0 showed a highest value of $7.9 \mu\text{m}/\text{s}$. The mean velocity for our *A. ferrooxidans* cultures is lower than the observed velocities between $20 \mu\text{m}/\text{s}$ and $45 \mu\text{m}/\text{s}$ for *E. coli* or *B. subtilis* [35, 44]. We believe that it is natural that these values are lower because for *A. ferrooxidans* the flagella may be also used for substrate adhesion, whereas in *E. coli*, for example, it is used for chemotaxis.

4 Conclusions

Three strains of *Acidithiobacillus ferrooxidans* – native, ATCC, and DSMZ – were cultured with different energy sources in an attempt to drive active locomotion on the cells. We used ferrous sulfate, elemental sulfur, and pyrite combinations. The *A. ferrooxidans* strain (DSMZ 24419) exhibited a phenotype associated with active locomotion that is expressed in the presence of elementary sulfur. This phenotype was not expressed in the other strains, thereby indicating a large genetic variability between the strains of *A. ferrooxidans*. We also believe that the absence of flagellar mobility may be also due to the domestication of commercial strains. The presence of flagella in the DSMZ enforces previous studies regarding the activation of genes for adhesion in the presence of the mineral. The swimming bacteria undergo different oxidation routes, which we followed by measuring pH, Eh, and the concentration of cells, ferrous, and sulfates ions as a function of time. In the presence of iron, swimming DSMZ cultures accommodate for the iron oxidation kinetics even if grown in a sulfur-rich environment. We found a distinctive correlation between the active locomotion and the pH of the culture, where low values inhibit the swimming stage.

In this work, we activate swimming locomotion in a particular strain of *A. ferrooxidans* and we followed the behavior of the culture during oxidation. We used pH, Eh, and the concentration of cells and ions as variables that quantify and qualify the status of the bacterial culture. More importantly, we identified them as central variables that can be used to control and engineer the motion of cells. The identification of the proper energy source and the preferred metabolic path of active-capable bacteria are essential for energy efficient biotechnological platforms. For instance, activating swimming locomotion provides routes for bio-remediation reactor design where flagella activation and control, through pH, can be used towards enhanced culture bio-mixing.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflicts of interest.

Research involving human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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