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Characterisation of growth parameters for the extremely acidophilic archaeon *Ferroplasma acidiphilum* DSM 12658 using a two-step turbidimetric growth measurement method ☆

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ABSTRACT

The acidophilic archaeon *Ferroplasma acidiphilum* exhibits remarkable adaptations to life in highly acidic environments. Nevertheless, the investigation of its molecular biology is challenging because of the slow growth of the organism, low biomass yield, and limitations of standard growth measurement techniques caused by iron oxidation.

- A novel two-step turbidimetric growth measurement (2TGM) method was developed to address the shortcomings associated with iron precipitation in culture media. The method comprises two distinct preparation steps.
- The initial 2TGM step involves centrifuging culture samples at high speed to remove *F. acidiphilum* cells and iron precipitates, thus preparing a blank.
- The second 2TGM step involves centrifuging the culture at a lower speed with the objective of selectively removing iron precipitates, thus allowing *F. acidiphilum* cells to remain suspended in the supernatant. This preparation enables subsequent measurement of cell density.

A study on the growth of *F. acidiphilum* showed a standard logistic pattern with a 35-h lag phase, approximately 9-h doubling time, and 0.042 OD carrying capacity. A new two-step turbidimetric growth measurement (2TGM) method overcomes limitations of existing approaches, enabling further investigation of *F. acidiphilum*.

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Method details

Background

Ferroplasma acidiphilum, an extremophilic archaeon that thrives in highly acidic, iron-rich environments, was isolated from a bioleaching pilot plant [1]. Notably, the cells lack a cell wall, resulting in a pleomorphic (irregularly shaped) morphology [1]. *F. acidiphilum* is a chemolithoautotroph that utilises the oxidation of ferrous iron (Fe^{2+}) and pyrite as a source of energy and fixes carbon dioxide as its sole carbon source [1]. The distinctive metabolic capabilities and adaptations of *F. acidiphilum* to extreme conditions offer insights into the limits of life on Earth and the potential for life to exist in similarly challenging environments [1–3]. This is of great value in understanding the origins of life. However, studying *F. acidiphilum* at the molecular level presents significant challenges. Its slow growth rate and low biomass yield make it difficult to use standard microbiological techniques. In addition, the measurement of growth itself is complicated by the change in colour of the culture medium due to the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) by *F. acidiphilum* [1]. This Fe^{3+} precipitates and increases in concentration as growth progresses. These factors interfere with traditional turbidimetric methods for measuring growth, which rely on light scattering from cells. Therefore, we propose a novel method for monitoring the growth of *F. acidiphilum* using a two-step turbidimetric growth measurement (2TGM) method.

Method details

Strain and growth conditions of F. acidiphilum

F. acidiphilum DSM 12658 was obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany). The seed culture of *F. acidiphilum* was prepared in 300 mL of 874 medium [4] at pH 1.70 and incubated at 35 °C with shaking at 150 rpm for 7 days. Subsequently, 1 mL of the seed culture was inoculated into 300 mL of fresh 874 broth and incubated under the same conditions (35 °C, pH 1.70, 150 rpm shaking) for 15 days.

Two-step turbidimetric growth measurement (2TGM)

The cell density of *F. acidiphilum* was monitored every 24 h. The growth of *F. acidiphilum* was monitored by the two-step turbidimetric growth measurement (2TGM) method. The 2TGM method has two main steps: blank preparation and sample preparation. In the first step (blank preparation), 750 µL of culture was collected at each designated time point and centrifuged at 12,000 × g for 5 min to remove cells and precipitant (Fe^{3+}). 500 µL of the supernatant was used as a blank and transferred to a cuvette. In the second step (sample preparation), another 750 µL of the culture was collected and centrifuged at 500 × g for 10 min to sediment only the precipitant (Fe^{3+}), and then 500 µL of the supernatant containing *F. acidiphilum* cells was gently transferred to a cuvette. The optical density (OD) of both the blank and sample was measured at 600 nm using a spectrophotometer (GeneSys 20, Thermo Scientific, USA).

Determination of F. acidiphilum *cell density*

To validate the 2TGM method, cell density was determined in both blank and sample cultures. For the blank, 750 µL was collected at each time point, centrifuged at 12,000 × g for 5 min to remove cells and iron precipitate (Fe^{3+}), and 10 µL of the supernatant was applied to a glass slide. For the sample, 750 µL of culture was first centrifuged at 500 × g for 10 min to selectively pellet the iron precipitate. Then, 500 µL of the supernatant containing *F. acidiphilum* cells was transferred and centrifuged at 12,000 × g for 5 min. This second centrifugation step was crucial for concentrating the *F. acidiphilum* cells, as their density in the culture is typically low. The resulting cell pellet was resuspended in 30 µL of fresh 874 medium, and 10 µL of this concentrated suspension was applied to a glass slide. Cell density of *F. acidiphilum* for both blank and sample was observed using a phase-contrast microscope (BX43, Olympus, Germany) at 1000 × magnification. This differential centrifugation approach ensures that the blank is free of both cells and iron precipitates, while the sample retains *F. acidiphilum* cells.

Determination of F. acidiphilum *growth parameters*

The growth parameters of *F. acidiphilum*, including growth rate, doubling time, and carrying capacity, were determined using the logistic equation [5]. The analysis employed optical density (OD) data acquired via the 2TGM method and the Growthcurver package version 0.3.1 [6] in R software. The duration of the lag phase of *F. acidiphilum* was calculated on the basis of the logistic model using the miLAG package version 0.0.1 in R [7].

Method validation

Triplicate cultures of *F. acidiphilum* were grown and monitored using the 2TGM method (Table 1). Cell density of *F. acidiphilum* in the blank and sample cultures, as prepared by the 2TGM method, is illustrated in Fig. 1 and Fig. 2, respectively. The blank (Fig. 1) shows a consistent absence of *F. acidiphilum* cells over 360 h, confirming its stability. In contrast, the sample (Fig. 2) shows increasing

Table 1
Growth of *F. acidiphilum* measured using the 2TGM method.

No.	Time (h)	Optical density ($OD_{600\ nm}$)				
		Replicate 1	Replicate 2	Replicate 3	Average	SD
1	0	0.008	0.008	0.008	0.008	0.000
2	24	0.009	0.007	0.009	0.008	0.001
3	48	0.016	0.015	0.017	0.016	0.001
4	72	0.035	0.034	0.029	0.033	0.003
5	96	0.046	0.044	0.042	0.044	0.002
6	120	0.051	0.050	0.054	0.052	0.002
7	144	0.056	0.058	0.053	0.056	0.002
8	168	0.064	0.064	0.060	0.063	0.002
9	192	0.050	0.052	0.053	0.052	0.001
10	216	0.045	0.049	0.050	0.048	0.002
11	240	0.044	0.049	0.047	0.047	0.002
12	264	0.042	0.049	0.047	0.046	0.003
13	288	0.047	0.045	0.048	0.047	0.001
14	312	0.046	0.048	0.047	0.047	0.001
15	336	0.046	0.048	0.051	0.048	0.002
16	360	0.046	0.048	0.051	0.048	0.002

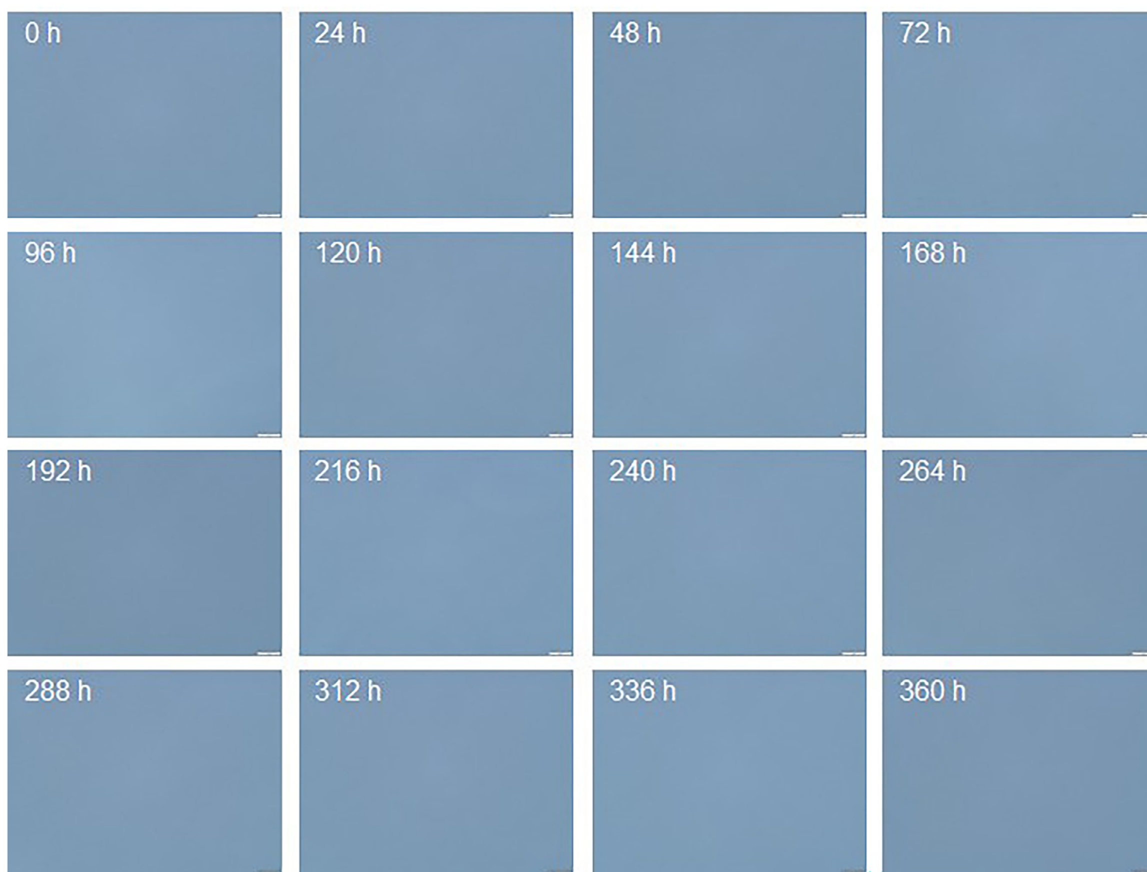

Fig. 1. Blank prepared by 2TGM method observed over 360 h. Phase-contrast micrographs at 16 time points show consistent absence of *F. acidiphilum* cells, demonstrating stability of the blank.

numbers of *F. acidiphilum* cells, with visible growth beginning at approximately 48 h. The 2TGM method effectively captures the growth dynamics of *F. acidiphilum* demonstrating its reliability for monitoring changes in cell density.

The average optical density (OD) of these cultures was used to generate the growth curve shown in Fig. 3. The calculated growth parameters are presented in Table 2.

The growth of *F. acidiphilum* was observed using the 2TGM method, which exhibited a typical logistic growth pattern with a lag phase of approximately 35 h (Table 2). Subsequently, the exponential phase was characterised by relatively fast growth with

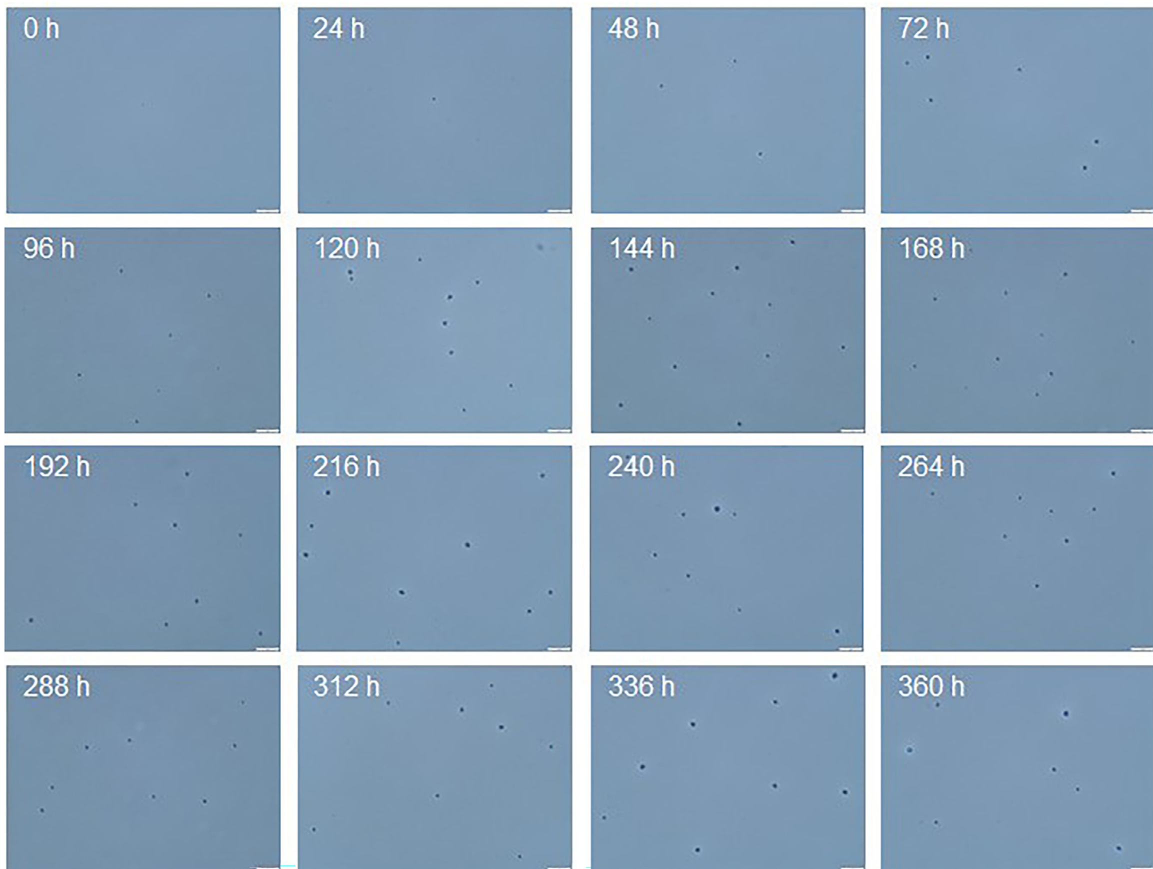

Fig. 2. Sample prepared by 2TGM method observed over 360 h. Phase-contrast micrographs at 16 time points show increasing *F. acidiphilum* cell numbers, with visible increase starting around 48 h.

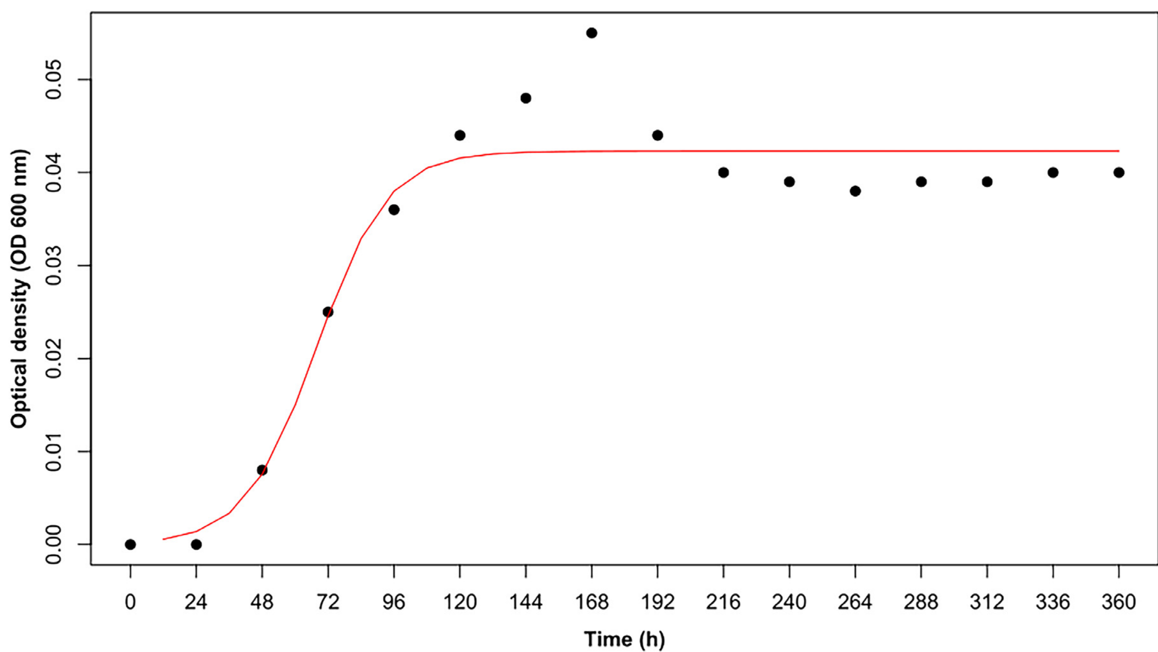

Fig. 3. Growth curve of *F. acidiphilum* measured using the 2TGM method.

Table 2
Growth parameters of *F. acidiphilum* estimated using the logistic equation.

No.	Parameters	Value
1	Lag phase (h)	35
2	Doubling time (h)	8.980
3	Carrying capacity (OD)	0.042
4	Standard error of the carrying capacity	0.001
5	*p* value of the carrying capacity	2.22×10^{-13}
6	Growth rate (r)	0.077
7	Standard error of the growth rate	0.026
8	*p* value of the growth rate	0.010
9	Residual SE from nonlinear least squares fit of the model to the data (sigma)	0.005
10	Degrees of freedom	13
11	Time at the inflexion point of the logistic curve (h)	67.752
12	Area under the curve of the fitted logistic equation	12.359
13	Area under the curve of the measurements	12.360

a doubling time of approximately 9 h (Table 2), reaching a maximum cell density of 0.042 OD (carrying capacity) (Table 2). The inflexion point at approximately 68 h (Table 2) marked the transition to the stationary phase. Furthermore, the close alignment between the areas under the curve for the fitted (12.359) (Table 2) and measured (12.360) (Table 2) growth profiles validates the accuracy of the model in representing the growth of *F. acidiphilum*.

In conclusion, the 2TGM method is an effective tool for determining the growth parameters of *F. acidiphilum*. The applicability of this method extends to the characterisation of the growth of other microorganisms that use nutrients in culture media and cause changes in their properties.

Limitations

The 2TGM method requires freshly prepared 874 medium for the cultivation of *F. acidiphilum*. This is because the pH of the medium is a crucial factor in regulating the growth of this organism. Over time, the pH of the stored 874 medium can undergo significant fluctuations, which may result in inaccurate growth measurements.

Ethics statements

This study did not involve human or animal subjects. The authors declare that this manuscript is original and has not been published elsewhere.

Credit author statement

Montri Yasawong: Conceptualisation, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision. **Thunwarat Songngamsuk:** Investigation, Validation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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