Investigating the growth kinetics of acidophiles isolated from arsenic-bearing waste

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INTRODUCTION

Acidithiobacillus ferroxidans is a chemolithoautotrophic bacterium which oxidize iron and sulfur, playing a significant role in bioleaching and bioremediation processes.
Characteristics: Rod-shaped bacterium that occurs as single cells or in the form of chains. It is an autotroph, meaning it produces carbon dioxide metabolites using inorganic substrates. It primarily oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) and reduces sulfur compounds (e.g., thiosulfate and elemental sulfur) as energy sources, according to the following reactions:

$4Fe^{2+} + 4H^+ + O_2 \xrightarrow{A.\ ferrooxidans} 4Fe^{3+} + 2H_2O$	• substrate oxidation, • proton consumption, • pH increase
$4Fe^{3+} + 4H_2O \rightarrow 4Fe(OH)^{2+} + 4H^+$ $4Fe(OH)^{2+} + 4H_2O \rightarrow 4Fe(OH)_2^{+} + 4H^+$ $4Fe^{3+} + 4H_2O \rightarrow 2Fe_2(OH)_2^{4+} + 4H^+$ $2Fe_2(OH)_2^{4+} + 8H_2O \rightarrow 4Fe(OH)_3 + 8H^+$	• Fe^{3+} increase • Series hydrolysis reaction of Fe^{3+} compounds • pH decrease
$3Fe(OH)_3 + 4SO_4^{2-} + 3Fe^{3+} + 3H_2O + 2NH_4+ \rightarrow 2M[Fe_3(SO4)_2(OH)_6]\downarrow + 3H^+$ $M = K^+, H_3O^+, NH_4^+$	• jarosite precipitation $2M[Fe_3(SO4)_2(OH)_6]\downarrow$

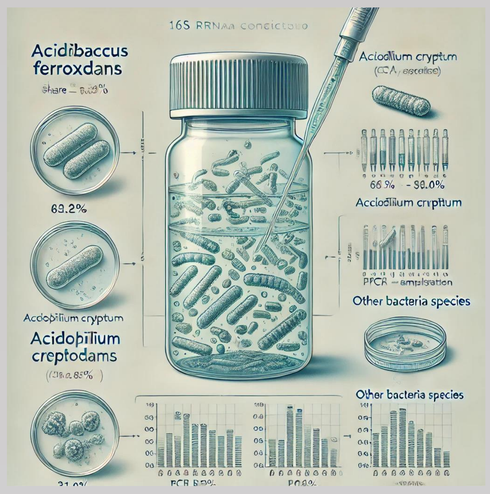
Ecological role: It thrives in highly acidic environments, with an ideal pH range of 2 to 3, making it indispensable in managing acidic mine drainage. The organism plays a pivotal role in facilitating the leaching of metals from insoluble sulfide and oxide ores, both in natural settings and in industrial mineral extraction. *A. ferroxidans* is key to the biogeochemical cycling of iron and sulfur in nature. Its activity contributes to the formation of acid mine drainage, where sulfide mineral oxidation increases acidity and enhances metal solubility. This bacterium is employed in bioleaching processes to extract metals such as copper, gold, and uranium by breaking down their ore matrices through its metabolic activities.

Applications: *A. ferroxidans* is utilized in bioleaching processes to extract valuable metals from low-grade ores and mining waste, offering a more sustainable alternative to traditional chemical leaching methods. Additionally, it effectively treats contaminated environments, especially those polluted by heavy metals and acidification, playing a key role in stabilizing and rehabilitating such areas.

Research significance: *A. ferroxidans* is a model organism for studying extremophiles, microbial ecology in acidic environments, and the biochemical mechanisms of metal oxidation. Ongoing research aims to improve bioleaching processes and enhance metal recovery efficiency.

Microorganisms under investigation

A consortium of acidophilic bacteria has been **isolated from arsenic-containing waste at the Złoty Stok site in Lower Silesia**, which is intended for use in this the presence project. Analysis of the 16S rRNA gene sequences indicated that ***Acidithiobacillus ferrooxidans*** was the most prevalent species, comprising **68.2%** of the community, followed by **31.0%** from the ***Acidophilum cryptum*** group, with representing **other species**. This finding underscores **0.8%** of acidophilic microorganisms in former mining regions, suggesting that toxic ions could be released in acidic conditions.



GOAL OF THE RESEARCH

- Investigating the bacteria consortium growth with the ferrous substrate in relation to bacterial cell biomass, substrate, product, and Zeta potential monitoring.
- Determining specific growth rates and yield coefficients for biomass and product formation.
- Identification of kinetic growth parameters.

METHODOLOGY

Basal culture medium, 9K with composition (per L): $FeSO_4 \cdot 7H_2O$ 44.8 g, $(NH_4)_2SO_4$ 3.0 g, K_2HPO_4 0.5g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.1 g, $Ca(NO_3)_2$ 0.01 g.

Basal conditions: medium to flask capacity proportion: 1:5, 150 rpm, and 32 °C.

Inoculum preparation: The bacterial consortium isolated from arsenic waste originating from the Złoty Stok site at Lower Silesia was cultivated in a 9K medium using basal conditions for 24 h. Such culture was used for inoculation media in further experiments.

Kinetic growth at specific Fe^{2+} concentration: The process was conducted in a set of 250 mL flasks containing 50 mL of 9K medium. The medium inoculated with a constant amount of bacteria 8.7 ±1.7 mg was cultivated in the basal conditions. At a specific time, one culture flask was filtered through a Wathman No.1 double-layer filter (pore size 11 µm) to separate precipitated minerals from the cells. The filtrate was analyzed for Fe^{2+} and Fe^{3+} content with titration method [1]. The filtrate followed the proposed in [2] procedure with some authors modifications. In the final step, the bacteria biomass was suspended in 2 mL of pH 3.0 water, and measurements of biomass content and a Zeta potential […] were determined. The mass of bacteria was determined based on absorbance at 500 nm using a standard curve for the dry weight mass of consortium bacteria. Zeta potential of bacterial cells was measured for 24h culture in pH 2.0 and constant ionic strength 10^{-3} KCl using Zetasizer 2000 (Malvern, UK). Similar experiments were carried out for the $FeSO_4 \times H_2O$ substrate with a concentration range between 1 and 24 g/L of Fe^{2+} ions in the time of 0-48 hours.

[1] Acta Montanistica Slovaca, Volume 28 (2023), 4; DOI: 10.46544/AMS.v28i4.10
[2] Biochemical Engineering Journal, Volume 152 (2019), 15; DOI:doi.org/10.1016/j.bej.2019.107360

A. ferroxidans consortium cultivation in basal medium under basal conditions

filtration

Bacteria cells rich filtrate → Fe^{2+}, Fe^{3+} analysis

centrifugation

Bacteria pellet

rinsing

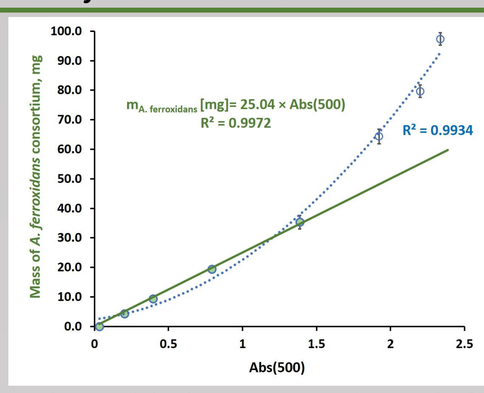
Bacteria suspension

Bacteria mass, Zeta potential analysis

RESULTS

Standard curve for dry mass of *Acidithiobacillus ferroxidans* consortium determination.

- Weight-drying method
- Measurements of absorbance at λ = 500 nm for dry mass in the range of 4.3 to 97.3 mg.
- Exponential fitting with $R^2 = 0.9934$.
- Linearity fitting with $R^2 = 0.9972$ between Abs(500) ranging from 0.2 to 1.4.

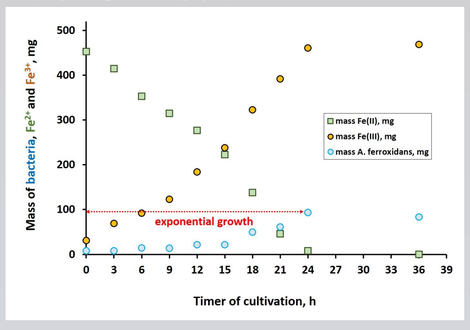


Relation of measured absorbance at 500 nm to dry mass of *A. ferroxidans*.

Batch cultivation of *A. ferroxidans* consortium, specific growth rate, yield coefficients

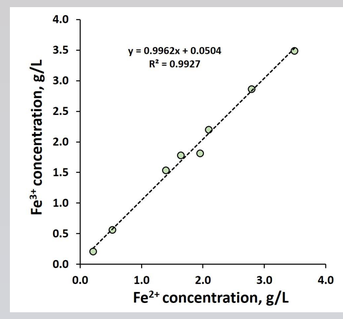
$$\frac{dX}{dt} = \mu \cdot X \Rightarrow X(t) = X_0 \cdot e^{-\mu \cdot t}$$

X – bacterial mass [mg]; X_0 – an initial mass of bacteria [mg];
t – time of the bacteria growth [h];
µ – **specific growth rate** [h^{-1}] dependent on substrate concentration.

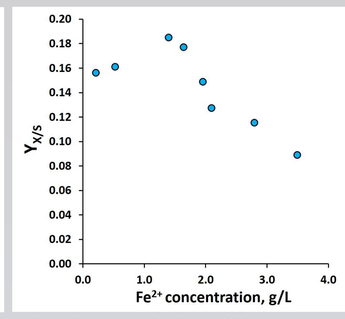


Graphical presentation of the mass [mg] changes of bacteria, substrate Fe^{2+} and product Fe^{3+} in the culture of *A. ferroxidans* cultivated on the $FeSO_4$ with **initial Fe^{2+} mass 453 mg** (3.8 g/L of Fe^{2+} ions).

$Y_{X/S}$, $Y_{P/S}$ ***Y*** **– yield coefficients** [-]; X/S – mass of cells (X) per unit mass of substrate (S), P/S – mass of product (P) per unit mass of substrate (S);



All of the substrate is converted into Fe^{3+}, according to stoichiometry of the reaction oxidation Fe^{2+} by *A. ferroxidans*.



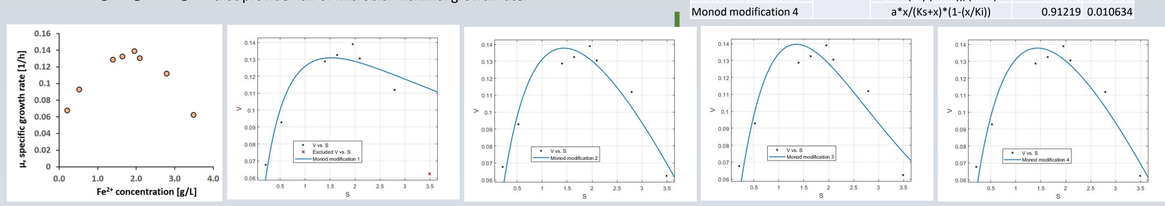
The biomass yield coefficient is not constant; initially increases for three beginning Fe(II) concentrations, then decreases, indicating bacteria cell inhibition when the $[Fe^{2+}] > 2$ g/L.

Monod model

$$\mu = \frac{\mu_{max} \cdot [Fe^{2+}]}{K_s + [Fe^{2+}]}$$

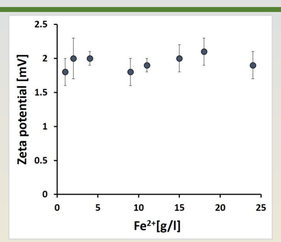
µ – **specific growth rate** [h^{-1}] dependent on substrate concentration; μ_{max} – maximal growth rate of bacteria; Ks – substrate concentration that provide half of microbial maximal growth rate.

Fit name	Data	Fit type	R-square	RMSE
Monod modification 1	V vs. S	a*x/(Ks+x+(x^2/Ki))	0.91352	0.009351
Monod modification 2		a*x/(x+Ks)*(1-Ki*x)	0.91219	0.010634
Monod modification 3		a*x*(exp(-Ki*x))/(Ks+x)	0.85875	0.013487
Monod modification 4		a*x/(Ks+x)*(1-(x/Ki))	0.91219	0.010634



Experimental determination of dependency µ = f(Fe^{2+}) (V=f(S)) showed an inhibition effect on specific growth rate with increasing substrate concentration. All applied models concerning substrate inhibition do not provide sufficient fit to the experimental results. The inhibition model is more complicated and should also consider the product inhibition effect.

Zeta potential



Graphical presentation dependency of the initial substrate (Fe2+) concentration vs. Zeta potential measured for the *A. ferroxidans* consortium in 24th-hour growth. **The Zeta potential is relatively constant with average value of 1.94 ± 0.11 [mV] for all measured samples,** there is no influence of Fe(II) content in the culture medium on bacterial net surface chargé.

SUMMARIZATION AND CONCLUSIONS

Microorganisms isolated from natural sources in the form of mixed cultures are useful tools for biotechnological processes. Depending on the isolation environment, mixed cultures have their own unique properties to ensure high natural process efficiency. Therefore, learning about their properties is important for use in controlled biotechnological processes. This presentation presents the characterization of a consortium of extremophilic bacteria for which the natural habitat is low pH conditions (pH 2-3) isolated from arsenic-containing waste. The bacteria were cultured on 9K liquid medium and the isolation procedure was carried out. The considerable difficulty in isolating pure bacterial cultures is complicated by the solid product formed, mainly jarosite, which is isolated by filtration and centrifugation with bacteria cells. Thus, effective reliable isloation method has to be developed. The growth of consortium containing A. ferroxidans is typical for bacteria, wich counterpart with substrate consumption, biomass and product formation. As it is known in the literatur, the inhibition effect of bacteria growth when th substrate concentration increases. This is reflected in both specific growth rate and biomass coefficient. The models concerning substrate inhibition solely are insufficient to provide reliable inhibition parameters. Therefore, the model has to be extended for the product inhibition effect. The Zeta potential was also measured to provide the nformation about the influence of substrate concentration on chargé which is on the surface of the bacteria cell surrface which is important for future experiments concerning application of the bacteria in various metal leaching processes.

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