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Samir Daniel, Anne-Gwenaëlle Guezennec, Agathe Hubau, Douglas O. Pino-Herrera, Eric Olmos. Impact of gas-liquid mass transfer and power dissipation on *Acidithiobacillus ferrooxidans* sulfur oxidation and growth in shake flasks. 14th European Congress of Chemical Engineering and 7th European Congress of Applied Biotechnology (ECCE and ECAB), DECHEMA e.V.; European Federation of Chemical Engineering (EFCE); European Society of Biochemical Engineering Sciences (ESBES), Sep 2023, Berlin (Germany), Germany. hal-04190464

**HAL Id: hal-04190464**

**<https://brgm.hal.science/hal-04190464v1>**

Submitted on 29 Aug 2023

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# Impact of gas-liquid mass transfer and power dissipation on *Acidithiobacillus ferrooxidans* sulfur oxidation and growth in shake flasks.

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## Context and purpose

*At. ferrooxidans*<sup>1</sup> is a chemolithoautotrophic bacteria that can oxidize elemental sulfur S<sup>0</sup> in the presence of oxygen and carbon dioxide according to this equation<sup>2</sup>:  $2 S^0 + 3 O_2 + 2 H_2O \rightarrow 2 SO_4^{2-} + 4 H^+$ . Kinetics of sulfur bio-oxidation has been studied<sup>3,4</sup> in shake flasks, but none of these studies has considered if the agitation and aeration conditions applied to these cultures were sufficiently high to avoid O<sub>2</sub> limitation and to promote efficient sulfur particles dispersion. Our aim is to study the impact of both gas-liquid mass transfer and power dissipation per unit of volume on *At. ferrooxidans* S<sup>0</sup>-oxidising activity and growth in shake flasks.

## Material and methods

*At. ferrooxidans* DSMZ 14882 was sub-cultured for three months with 1% (wt/vol) S<sup>0</sup> and 0Km medium<sup>5</sup>. Autoclaved shake flasks were inoculated with 10% of a previous culture, 89% of fresh medium and 1% of sterile S<sup>0</sup>. Then, they were incubated at a temperature of 30°C for 10 days in various agitation and aeration conditions (see Table 1). Gas liquid-mass transfer coefficient, power dissipation and critical agitation rate ensuring particle suspension were determined using previously developed models<sup>6,7</sup>.

Table 1: Aeration and agitation conditions applied to the cultures.

Condition	V (mL)	D <sub>o</sub> (cm)	N (rpm)	N <sub>c</sub> (rpm)	k <sub>LA</sub> (h <sup>-1</sup> )	P <sub>o</sub> /V (W/m <sup>3</sup> )
1	75	0.019	<b>350</b>	<b>232</b>	<b>41</b>	<b>982</b>
2	150	0.019	<b>350</b>	<b>311</b>	<b>23</b>	<b>619</b>
3	75	0.025	195	217	<b>23</b>	<b>201</b>
4	150	0.019	230	311	<b>14</b>	<b>198</b>
5	75	0.051	101	181	<b>14</b>	<b>34</b>
6	150	0.051	101	243	<b>8.0</b>	<b>21</b>
7	100	n.a	n.a	n.a	<b>0.0</b>	<b>0.0</b>

k<sub>LA</sub>: gas-liquid transfer coefficient ; P<sub>o</sub>/V: power dissipation per unit of volume ; V: culture volume ; D<sub>o</sub>: incubator orbit diameter ; N: agitation rate ; N<sub>c</sub>: critical agitation rate for S<sup>0</sup> particles suspension.

Bacterial concentration and pH were monitored daily while dissolved sulfate concentration was measured by IEC at the beginning and at the end of the culture.

Oxygen sensor spots (PreSens) were used to measure dissolved oxygen concentrations and experimental  $k_{La}$ .

## Results and discussion

*At. ferrooxidans* growth was improved with the increase of  $k_{La}$  and  $P_0/V$  (Figure 1). Higher  $k_{La}$  and  $P_0/V$  fastened  $S^0$ -bio-oxidation kinetics as well (data not shown). Moreover, whatever the operating conditions used (except condition 7 for which no measurement was carried out), dissolved oxygen concentration was always above 85% of saturation, indicating there was no limitation of the gas-liquid mass transfer.

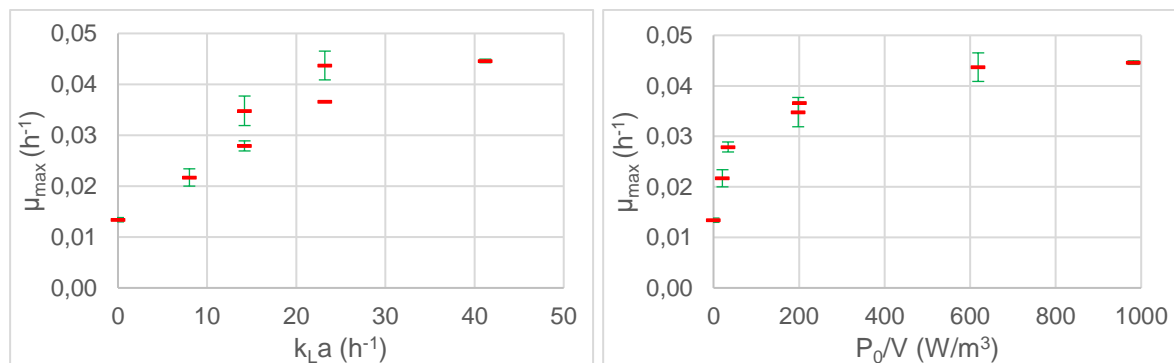


Figure 1 a (on left) & b (on right): Maximum bacterial growth rate depending on  $k_{La}$  (a) and  $P_0/V$  (b). Error bars represent standard deviation.

With the same  $P_0/V$  but different  $k_{La}$  (conditions 3 and 4),  $\mu_{max}$  remained the same. On the contrary, for a given  $k_{La}$  value,  $\mu_{max}$  was increased when  $P_0/V$  was also increased (conditions 2-3, 4-5). Interestingly,  $\mu_{max}$  reached a plateau (conditions 1 & 2), when the agitation rate was higher than the critical agitation rate ( $N_c$ ) for complete suspension of sulfur particles in the liquid. Thus, these results seem to indicate that microbial growth of *At. ferrooxidans* in OKm medium was mainly influenced by  $S^0$  particles dispersion and not by gas-liquid mass transfer.

## Conclusion

Operating conditions ( $N$ ,  $D_0$ ,  $V$ ) change the aeration and homogenization of the culture. Those parameters need to be considered to avoid limiting conditions and promote bacterial growth. Two key factors for *At. ferrooxidans*  $S^0$ -bio-oxidation in shake flasks have been identified:  $P_0/V$  and  $N_c$  which determine the sulfur particles dispersion and suspension.

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