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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*¹ is a chemolithoautotrophic bacteria that can oxidize elemental sulfur S⁰ in the presence of oxygen and carbon dioxide according to this equation²: $2 S^0 + 3 O_2 + 2 H_2O \rightarrow 2 SO_4^{2-} + 4 H^+$. Kinetics of sulfur bio-oxidation has been studied^{3,4} 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 O₂ 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* S⁰-oxidising activity and growth in shake flasks.

Material and methods

At. ferrooxidans DSMZ 14882 was sub-cultured for three months with 1% (wt/vol) S⁰ and 0Km medium⁵. Autoclaved shake flasks were inoculated with 10% of a previous culture, 89% of fresh medium and 1% of sterile S⁰. 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^{6,7}.

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

Condition	V (mL)	D _o (cm)	N (rpm)	N _c (rpm)	k _{LA} (h ⁻¹)	P _o /V (W/m ³)
1	75	0.019	350	232	41	982
2	150	0.019	350	311	23	619
3	75	0.025	195	217	23	201
4	150	0.019	230	311	14	198
5	75	0.051	101	181	14	34
6	150	0.051	101	243	8.0	21
7	100	n.a	n.a	n.a	0.0	0.0

k_{LA}: gas-liquid transfer coefficient ; P_o/V: power dissipation per unit of volume ; V: culture volume ; D_o: incubator orbit diameter ; N: agitation rate ; N_c: critical agitation rate for S⁰ 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.

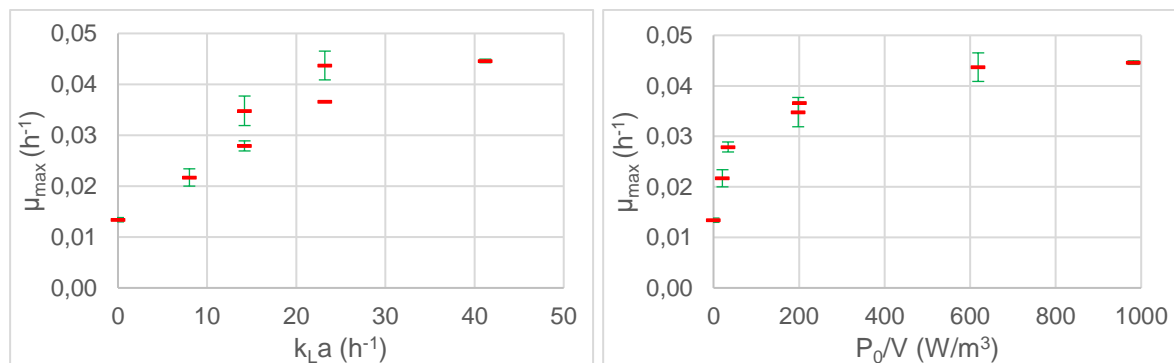


Figure 1 a (on left) & b (on right): Maximum bacterial growth rate depending on k_{La} (a) and P_0/V (b). Error bars represent standard deviation.

With the same P_0/V but different k_{La} (conditions 3 and 4), μ_{max} remained the same. On the contrary, for a given k_{La} value, μ_{max} was increased when P_0/V was also increased (conditions 2-3, 4-5). Interestingly, μ_{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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