Master's Thesis Engineering Technology

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The influence of copper and silver ions on Cupriavidus metallidurans biofilm formation and development

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INTRODUCTION

Biofilm formation can cause bacterial resistance and water spoilage. Aboard the International Space Station (ISS), *Cupriavidus metallidurans* strains, such as NA4, have been discovered despite the presence of decontaminants such as silver. Another strain well known for its metal resistance is *C. metallidurans* CH34. Both strains are able to form biofilms. Biofilms are matrices of cells and extracellular polymeric substances (EPS) in association with a solid surface [1-3].

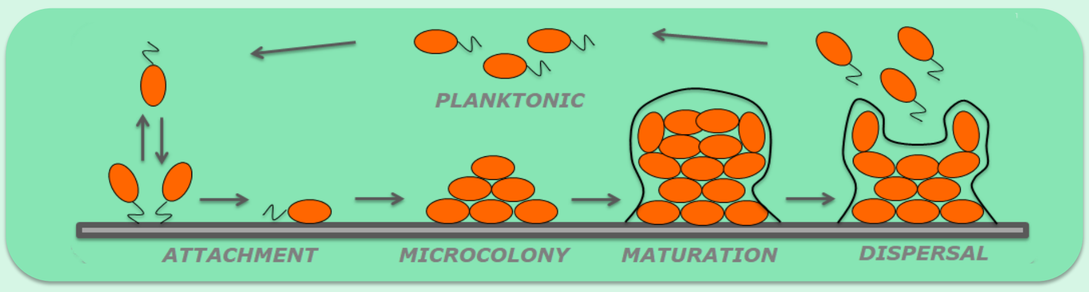


Figure 1: Biofilm formation and development

METHODS

Several experimental setups have been used to determine copper and silver influence on *Cupriavidus metallidurans* CH34 and NA4 biofilm development and formation in 96-well plates.

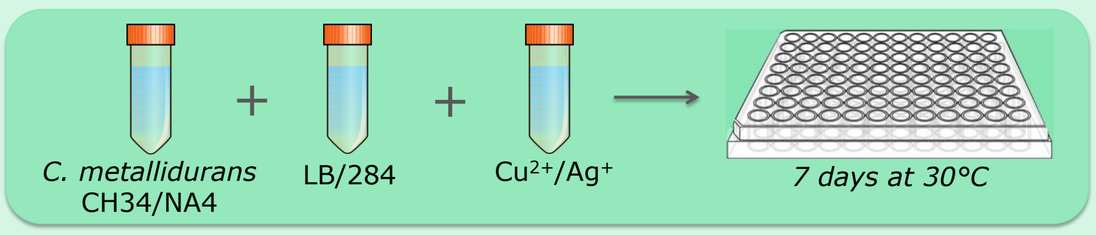


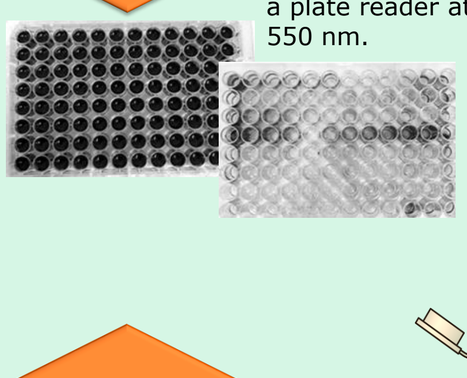
Figure 2: 96 well plate assay for the development of *C. metallidurans* biofilms

Optical Density (OD)

As an indication for bacterial growth, the OD of bacterial suspensions was measured using a plate reader at 600 nm.

Crystal Violet (CV) staining

Biofilms were stained using CV. Afterwards, the CV was solubilised by using acetic acid and quantified using a plate reader at 550 nm.

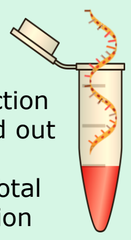


SCANNING ELECTRON MICROSCOPY (SEM)

SEM was performed to study structural biofilm changes. Biofilms were grown in an MBEC™ Biofilm Inoculator.

RNA EXTRACTION

RNA extraction was carried out using the Promega Total RNA Isolation System.



RESULTS

The influence of copper and silver ions on biofilms

Figure 1 shows the CV/OD values of *C. metallidurans* NA4 (biofilm formation normalized to planktonic cell density) in 284 medium in the presence of copper.

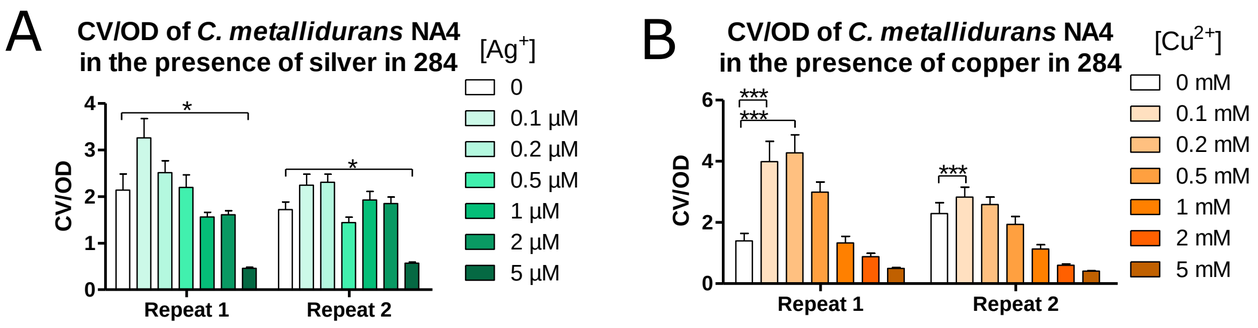


Figure 3: Ratio of CV value to OD value for *C. metallidurans* NA4 grown in 284 medium appended with A) $AgNO_3$ and B) $CuSO_4$

Table 1 gives an overview of all observed effects of copper and silver on both *C. metallidurans* CH34 and NA4. Both stimulating and inhibiting concentrations are shown.

Table 1: A summary of the optimal and inhibiting concentrations of copper and silver ions on *C. metallidurans* CH34 and NA4 biofilm growth in 284 medium

	[Cu²⁺]		[Ag⁺]	
***C. metallidurans* strain**	**Stimulation**	**Inhibition**	**Stimulation**	**Inhibition**
CH34	1 – 2 mM	5 mM	/	5 µM
NA4	0.1 – 0.2 mM	2 – 5 mM	0.1 – 0.2 µM	5 µM

Scanning electron microscopy (SEM)

Table 2: *C. metallidurans* CH34 and NA4 biofilm characteristics observed by SEM imaging

	C. metallidurans CH34	C. metallidurans NA4
EPS formation	++	+
Cell clustering	+	++
Biofilm formation	+	++

(+ = observed, ++ = observed to a greater extent)

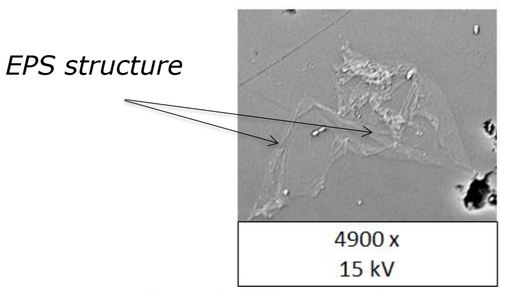


Figure 4: SEM image of C. metallidurans CH34 biofilm grown in 284

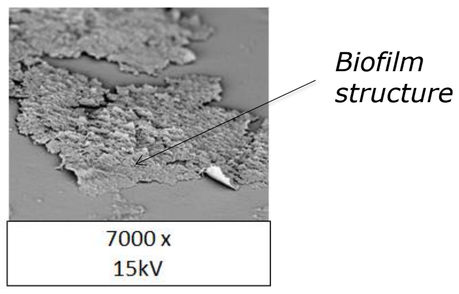


Figure 5: SEM image of C. metallidurans NA4 biofilm grown in 284

CONCLUSIONS

- **Silver** has an **inhibiting effect** on *C. metallidurans* CH34 and NA4 planktonic and biofilm growth at 5 µM.
- **Copper** has an **inhibiting effect** on *C. metallidurans* CH34 and NA4 bacterial and biofilm growth at 5 mM, but has a **stimulating effect** on *C. metallidurans* CH34 at **1 mM and 2 mM**, and on *C. metallidurans* NA4 at **0.1 mM and 0.2 mM**.
- *C. metallidurans* NA4 forms more biofilm mass in comparison to CH34 and has a less developed EPS structure and a more layered and dense cell cluster composition.

REFERENCES

[1] K. Mijnendonckx et al., "Characterization of the survival ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from space-related environments," *Microb Ecol*, vol. 65, no. 2, pp. 347-60, 2013.
[2] P. J. Janssen et al., "The complete genome sequence of *Cupriavidus metallidurans* strain CH34, a master survivalist in harsh and anthropogenic environments," *PLoS One,* vol. 5, no. 5, 2010.
[3] H. C. Flemming, J. Wingender, U. Szewzyk, P. Steinberg, S. A. Rice, and S. Kjelleberg, "Biofilms: an emergent form of bacterial life," *Nat Rev Microbiol*, vol. 14, no. 9, pp. 563-75, 2016.

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