Growth and biofilm formation of *Cupriavidus metallidurans* CH34 on different metallic and polymeric materials used in spaceflight applications

Nissem Abdeljelil[a,b,c,d]*, Najla Ben Miloud Yahia[d], Ahmed Landoulsi[c], Abdelwaheb Chatti[c], Ruddy Wattiez[a], Rob Van Houdt[b] and David Gillan[a]

[a]*Proteomics and Microbiology Lab, Research Institute for Biosciences, Mons University, Mons, Belgium;* [b]*Microbiology Unit, Interdisciplinary Biosciences, Belgian Nuclear Research Centre, SCK CEN, Mol, Belgium;* [c]*Laboratory of Biochemistry and Molecular Biology, Faculty of Sciences of Bizerte, University of Carthage, Jarzouna, Tunisia;* [d]*National Center for Nuclear Sciences and Technologies, Sidi Thabet, Tunisia*

*Nissem.abdeljelil@student.umons.ac.be

Growth and biofilm formation of *Cupriavidus metallidurans* CH34 on different metallic and polymeric materials used in spaceflight applications

Bacteria biofilm formation and its complications are of special concern in isolated structures, such as offshore stations, manned submarines and space habitats, as maintenance and technical support are poorly accessible due to costs and/or logistical challenges. In addition, considering that future exploration missions are planned to adventure farther and longer in space, unlocking biofilm formation mechanisms and developing new antifouling solutions are key goals in order to ensure spacecraft's efficiency, crew's safety and mission success. In this work, we explored the interactions between *Cupriavidus metallidurans*, a prevalently identified contaminant onboard the International Space Station, and aerospace grade materials such as the titanium alloy TiAl6V4, the stainless steel AISI 316 (SS316) and Polytetrafluoroethylene (PTFE) or Teflon. Borosilicate glass was used as a control and all surfaces were investigated at two different pH values (5.0 and 7.0). Biofilms were almost absent on stainless steel and the titanium alloy contrary to Teflon and glass that were covered by an extensive biofilm formed via monolayers of scattered matrix-free cells and complex multilayered clusters or communities. Filamentous extracellular DNA structures were observed specifically in the complex multilayered clusters adherent to Teflon, indicating that the employed attachment machinery might depend on the physicochemical characteristics of the surface.

Keywords: Cupriavidus metallidurans; biofilm; eDNA; PTFE; stainless steel; titanium alloy

Introduction

Bacteria have the ability to attach to a surface and/or to each other and to form multicellular communities embedded in a polymeric matrix known as biofilms (Stoodley et al., 2002). This phenomenon is linked to an increased resistance to antibacterial agents and despite decontamination processes, adherent bacteria are problematic for many industries such as health care, drugs manufacturing, food

processing, oil extraction and electronics (Harrison et al., 2007; Mah and O'Toole, 2001; Van Houdt and Michiels, 2010). Biofilm formation and its complications are of special concern in isolated structures, such as offshore stations, manned submarines and space habitats, as maintenance and technical support are poorly accessible due to costs and/or logistical challenges. Biofilms can cause surface corrosion, hardware degradation, clots and failures in critical equipment such as piping or filters of life supporting systems, water-recycling units, radiators and in extravehicular mobility units (EMU). In fact, related to spaceflight applications, such incidents have already been observed in the Salyut, Mir and Skylab stations, and continue to be a challenging threat on board the International Space Station (ISS) (Diaz et al., 2019; Flemming, 1998; Klintworth et al., 1999).

The main concern for biofilm control strategies in spacecraft revolves around water storage and recycling systems as the initiation of biofilm formation depends largely on humidity (water droplets, vapor, condensate, water/liquid pipes, liquid tanks). For instance, numerous hazardous points have been identified in spacecraft wet systems as potential entries for microbial contamination or to be prone to biofilm formation, and most of them have been traced to the sanitary space, the Environmental Control and Life Support System (ECLSS) of the spacecraft or of its EMU and in thermal control systems (Roman and Minton-Summers, 1998; Schultz et al., 1991; Squire et al., 2014). In general, the ECLSS is a complex network of tanks, pumps, pipes, hoses and filters that allows the regeneration of potable water from the recovery of urine and humidity condensate in an enclosed environment. On the ISS, it is especially complicated and it extends on 29 orbital replacement units (ORU) where countless components made from various materials (e.g. Stainless Steel 302, 303, 304, 316, 318-8, 6061 Aluminum, TiAl6V4, Inconel 718) can be directly or indirectly interacting. Other materials of

spacecraft or space suits such as Teflon, Nafion, Fluorel, nylon, Nomex, Gore-tex, nickel and boron-nickel alloys, 60NiTi, graphite, silver, polyurethane coated aluminum and gold-anodized aluminum can also be in contact with humid environments (Squire et al., 2014).

Both urine and humidity condensate collected in spacecraft can contain a large number of molecules and various carbon sources that are suitable for microbial proliferation. Even after processing, water samples from the Mir station had 0.005 to 0.023 g L-1 of total organic carbon. Analysis of humidity condensate in Shuttle missions STS-45 and STS-47 showed that the total organic carbon levels ranged from 0.12 g L-1 to 0.7 g L-1 (National Research Council, 2000). These concentrations are sufficient for microbial proliferation, especially for oligotrophic bacteria that can survive at levels of carbons as low as 0.001 g L-1 (Ishida and Kadota, 1981). Next to water and available nutrients, the characteristic surface properties of the materials, such as hydrophobicity, surface energy and electron donor/acceptor potential, will play a crucial role in orienting microbial adhesion.

Squire et al. (2014) indicate that due to technical limitations, the routine antimicrobial procedure (gamma irradiation or extended heat treatment at 87.7°C) cannot be applied to all ORUs elements before launch. In fact, 5 from 16 items that are launched wet or containing water are not subjected to disinfection. This could create favorable conditions for inflight microbial growth and potential biofilm formation that could spread inside the wet system. Also microbial monitoring campaigns onboard the ISS showed recurrent microbial contamination events (Van Houdt and Leys, 2020; Zea et al., 2020). Although biofilms in water systems are interacting multispecies communities (Thompson et al., 2020; Yang et al., 2021), one species that attracted attention is *Cupriavidus metallidurans*. A Gram-negative bacterium belonging to the

Burkholderiaceae family that has been detected from 2009 to 2019 in almost all samples coming from the wastewater tank, the potable waterbus or the condensate (Mijnendonckx et al., 2013; Zea et al., 2020). This facultative chemolithotrophic motile microbe shows resistance to a broad range of metals, including silver used as disinfectant onboard ISS, and is able to adapt to various harsh conditions, including low nutrients environments (Maertens et al., 2020; Mijnendonckx et al., 2019; Mijnendonckx et al., 2013; Van Houdt et al., 2021; Zhang et al., 2018). In addition, bacteria are exposed to specific conditions (e.g. microgravity and cosmic radiation) during spaceflight (Acres et al., 2021; Bijlani et al., 2021; Horneck et al., 2010; Huang et al., 2018), which have also been studied for *C. metallidurans* type strain CH34 (Byloos et al., 2017; Leys et al., 2009) (De Gelder et al., 2009; Leroy et al., 2010). Furthermore, it is used to explore future spaceflight applications such as testing antimicrobial surfaces (Siems et al., 2022) as well as biomining and bioremediation (Byloos et al., 2017; Cockell et al., 2020; Santomartino et al., 2020). It is therefore a representative of the contaminant species found in humid spacecraft systems as well as a microbe with potential extra-terrestrial applications.

As future exploration missions are planned to adventure farther and longer in space, unlocking biofilm formation mechanisms and developing new antifouling solutions suitable for use in such a challenging environment are goals of key importance in order to ensure spacecraft's efficiency, crew's safety and mission success. In this work, we aim to explore the interactions between *C. metallidurans* and aerospace grade materials such as titanium alloy TiAl6V4, stainless steel AISI 316 (SS316) and polytetrafluoroethylene (PTFE) or Teflon. The impact of these materials on planktonic growth and biofilm formation, in addition to its biodeterioration effect are investigated at different pH values.

Material and methods

Bacterial strains, media and culture conditions

C. metallidurans CH34 was routinely grown in Tris–buffered mineral medium (MM284) (Mergeay et al., 1985) supplemented with 2 g L-1 sodium gluconate as the sole carbon source. The final pH was adjusted to 5.0 or 7.0 with HCl 37%. Although gluconate can chelate metal ions (Gyurcsik and Nagy, 2000) and could impact biofilm biomass and production of extracellular polymeric substances (Liu et al., 2021; Luo et al., 2019; Pal and Paul, 2013), it is readily consumed by *C. metallidurans* and as such would have a limited impact.

Preparation and setup of biofilm experiments

Materials tested included coupons of borosilicate glass (75 mm x25 mm x1 mm, stainless steel AISI 316 (used in ECLSS tanks and pipes, pumps and separators (Squire et al., 2014); 80 mm x 5 mm x 0.5mm), Teflon (used in hoses of thermal control systems, valves, bladders, insulation and gas separators (Squire et al., 2014; Wieland and Center, 1998); 18 mm x 13 mm x 3 mm) and titanium alloy TiAl6V4 (used in the outer shell of water tanks and tubing (Petala et al., 2020); 25mm x 25 mm x1 mm) were cleaned with deionized water and sonicated in 70% ethanol for 15 min. Next, coupons were rinsed with deionized water and autoclaved. Precultures of *C. metallidurans* CH34 were grown at 30°C on an orbital shaker (150 rpm) until an optical density (OD600) of 0.2 in MM284 (pH 7.0). Each sterilized coupon was then placed in a 50 mL conical centrifuge tube to which 15 mL of MM284 and 350 µL preculture were added. Tubes were then placed on a tilting (15°) shaker and incubated at room temperature (23 ± 2°C) and 1 rpm (turbulent flow conditions) for 168 hours.

Analysis of growth and biofilm formation

Planktonic growth was evaluated by measuring OD600 every 24 h. Biofilm formation was assessed using the crystal violet (CV) dye that binds to DNA and proteins. A solution of 0.1% CV was prepared (0.1 g of CV in 2 mL 95% ethanol and 98 mL of deionized water). At the end of the experiment, coupons were gently removed and softly rinsed 3 times with deionized water. Coupons were then air-dried and covered with the CV solution for 15 min. Afterwards, the CV solution was discarded and coupons were rinsed 3 times with deionized water. Coupons were left to air dry before quantifying the amount of CV by solubilizing in 4 mL of 95% ethanol and measuring absorbance at 620 nm. Measurements were normalized by subtracting the average of abiotic controls.

For biofilm viable counts, coupons were rinsed twice with sterile saline solution (0.85% NaCl). Next, coupons were placed in a new sterile tube with 15ml of saline solution and sonicated (30s, 35kHz) in a sonication bath (Elma, Germany) and subsequently vortexed (20 min, 2700 rpm). Cell suspensions (100 µL) of a serial ten-fold dilution in saline were spread on MM284 agar plates and incubated at 30°C. Colony forming units (CFU) were counted after 3 days.

Scanning Electron Microscopy (SEM)

Biofilms were examined under the SEM at the end of the experiment (168 h). Surfaces were gently rinsed with deionized water to remove non-adherent cells. Samples were then fixed for 2 hours in Bouin's solution (Jacobsen et al., 1980). Next, samples were dehydrated by immersion in an ethanol graded series as follows: 30 min in 70% ethanol, overnight in 70% ethanol, 30 min in 70% ethanol, 2 times 30 min in 90% ethanol, and 1 h in 100% ethanol. After dehydration, samples were dried with liquid CO2 in a critical point dryer. Finally, they were gold-coated in a sputter coater and placed in a JSM-7200F microscope (JEOL, Japan).

DNase treatment

Biofilms were grown as previously described (only at pH 7 to avoid interference with DNase activity). Growth medium was discarded and coupons were gently rinsed with sterile deionized water to remove loosely attached cells. Rinsed coupons were immersed in 3 ml of 5 µg mL-1 DNaseI (Qiagen, Germany) and incubated 1 h statically at 37°C. Next, samples were rinsed, stained with CV, dried at room temperature and observed with an automated inverted fluorescence microscope (TE2000-E; Nikon, Tokyo, Japan) equipped with a Cy5 filter set (excitation passband: 590-650 nm; emission: 660-740nm).

Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES)

The concentration of iron and aluminum leached from SS316 and TiAl6V4, respectively, were determined in the culture medium and the planktonic cells with ICP-AES. At the end of the experiment (168 h), coupons were removed from the culture medium and bacteria in suspension were pelleted via centrifugation at 10.000 g for 2 min. One mL of the supernatant was sampled and diluted with 14 mL of 5% HNO3. The pellets were resuspended in 1 mL of filtered deionized water and 2 mL of concentrated HNO3 was added to each sample and left to digest overnight at room temperature. Finally, 12 mL of 5% HNO3 was added. For abiotic controls, samples were centrifuged and 1 mL supernatant was diluted in 14 mL of 5% HNO3. All samples were stored at +5°C until analysis.

Contact-angle measurements

Coupons were incubated 3 h in sterile MM284 (final pH of 5 or 7). Next, coupons were removed from the medium and left to air-dry before analysis. Contact angle measurement was performed using a VCA optima goniometer (AST Products, Billerica,

USA) according to the static drop method. Three µL of deionized water were deposited on the dry substratum and six measurements were made for each sample. Images were analyzed with VCA Optima software (AST Products, Billerica, USA). The surface was considered hydrophilic or hydrophobic when the contact angle was less or more than 90°, respectively.

Statistical analysis

Statistical comparison of two samples was performed using the Student's t-test. Statistical comparison of multiple samples was performed using a one-way ANOVA analysis, followed by a post-hoc Tukey test. A p-value less than 0.05 was consider statistically significant.

Results and Discussion

Surface characterization

The surfaces of the uninoculated clean materials were first analyzed under the SEM. SS316 appeared regular and streaked with parallel thin bands (Figure 1b). Teflon appeared mainly smooth and crossed occasionally by stripes or stretches of nearly 5-µm width (Figure 1c). The glass surface was very smooth and free from any special topographic features (Figure 1a), whereas TiAl6V4 was rough and covered with crests and pits of 10 to 30 µm wide (Figure 1d). Contact angle measurements showed that glass was highly hydrophilic at pH 5.0 and 7.0, and no significant differences in contact angles were observed (Figure 2). Glass was the most hydrophilic substratum with very low contact angles that did not exceed 25.31 ± 6.45°. Teflon was the most hydrophobic material with contact angles not less than 109.11 ± 6.61° (Figure 2) and SS316 was amphiphilic. Finally, although contact angles of TiAl6V4 varied significantly between pH 5.0 and pH 7.0, it remained hydrophilic (Figure 2). Since the growth medium

(MM284) contained various ions and a carbohydrate (gluconate), contact angles were also measured in pure water. Indeed, MM284 components significantly decreased the contact angles for glass and TiAl6V4 at pH 5 and pH 7, and for SS316 at pH 5 (Figure 2). At the contrary, a significant increase in contact angle was observed for Teflon between the conditioning in MM284 pH 7.0 and in pure water.

Planktonic growth of C. metallidurans *CH34 in the presence of the test surfaces*

It is clear that bacteria show variable sensitivities to pH. Whereas neutrophilic bacteria prosper in circumneutral pH, acidophilic and alkaliphilic ones prefer opposite sides of the pH scale. In addition, the presence of macromolecules, nutrients and sensitizing compounds can change the bacterial response to the surrounding pH. Non-optimal pH or pH shifts are stressful for bacteria, it can unbalance the cellular homeostasis, disrupt membrane integrity and fluidity and inhibit key reactions involved in bacterial growth or survival (Guan and Liu, 2020; Padan et al., 2005). In addition, pH also plays a critical role in the readjustment of material surface properties by influencing hydrophobicity and electrostatic forces. It can slow or accelerate the corrosion of some materials and can enhance the leaching of their composing elements. Therefore, we performed the experiments at neutral (pH 7.0) and acidic (pH 5.0) conditions. Without the presence of the test surfaces *C. metallidurans* showed comparable growth at pH 5.0 and 7.0 (Figure 3). In addition, none of the test surfaces, and any leached elements, affected planktonic growth at pH 5.0 or 7.0 (Figure 3). Leaching of chromium and nickel ions, and aluminum and vanadium ions in biological media have been reported for stainless steel and titanium alloy, respectively (Berggren et al., 2004; Hedberg and Odnevall Wallinder, 2015; Herting et al., 2006; Mumme et al., 2005; Zhou et al., 2011).

Adhesion and biofilm formation of C. metallidurans *CH34 on the test surfaces*

Although prolonged contact with the test materials did not affect planktonic growth, the physicochemical differences between the test surfaces did significantly influence adhesion of *C. metallidurans* CH34. Crystal violet staining showed that Teflon and glass were more prone to *C. metallidurans* CH34 adhesion and biofilm formation than TiAl6V4 and SS316 (Figure 4, Supplementary Figure 1) with Teflon having significantly more adherent biomass than glass both in pH 5 and pH 7.0. Determination of viable biofilm cell numbers by plate count was also performed for glass and Teflon (pH 5), and corroborated the quantification by CV staining (Supplementary Figure 2). Adhesion to glass also varied according to the pH as the CV-stained adherent biomass on glass at pH 5.0 was nearly two-fold more important than at pH = 7.0. For Teflon, adhesion was independent of the pH. A previously reported theoretical model predicted that pH variations could influence S. aureus adhesion to glass because it alters its hydrophilicity/hydrophobicity (Hamadi et al., 2009). In our experiment, the surface properties of glass were not significantly affected during the experiment (Figure 2), therefore, other factors are putatively responsible for the observed difference between pH 5.0 and 7.0 (Sheng et al., 2008).

It is generally assumed that hydrophobic (reduces the strength of repulsion forces between the substratum and bacteria) and rough (creates areas of low shear stress) surfaces are more attractive for microbial adhesion than hydrophilic and smooth ones (Zheng et al., 2021). Indeed, the hydrophobic Teflon surface showed the most adhesion compared to the other materials. High adhesion on Teflon has previously been reported for *Pseudomonas aeruginosa* (Alfa et al., 2017), *Salmonella spp.* and *Listeria monocytogenes* (Sinde and Carballo, 2000). Although glass is very hydrophilic, with contact angles from 12.3 ± 2° to 25.3 ± 6.5°, it was the second most attractive material for *C. metallidurans* CH34 biofilm formation. The capacity of bacteria to attach both to

hydrophilic and hydrophobic surfaces has already been observed for *Staphylococcus epidermidis* and in *P. aeruginosa* (Cerca et al., 2005; Shelobolina et al., 2018), and was corroborated here for *C. metallidurans*.

Stainless steel 316, which was significantly more hydrophobic than glass (Figure 1), showed limited *C. metallidurans* CH34 biofilm formation at pH 5.0 and no biofilm formation at pH 7.0. (Figure 4). Interestingly, biofilm formation on stainless steel 304 in mineral water has recently been documented (Maertens et al., 2020). Studies demonstrated the superior capacity of SS316 over SS304 in repelling microbial adhesion in potable water (Percival et al., 1997). These differences could be explained by the different composition of SS316, which contains 2 to 3% of Molybdenum (Percival, 1999), or by specific growth conditions. In addition, SS316 is known to be less vulnerable to corrosion and pitting in the presence of chloride or low pH environments, and also to microbiologically influenced corrosion (Wang et al., 2021). The introduction of Mo in the alloy's formula has therefore significant consequences on the physicochemical properties of the material.

Titanium alloy contact angles 41.6 ± 5.7° to 53.8 ± 4.7° were significantly higher than glass. In addition, the alloy showed a rough surface rich in large pits that could have been ideal sites for bacterial cells seeking protection from shear forces. Nevertheless, almost no *C. metallidurans* CH34 biofilm formation was detected (low quantification with large deviations). However, TiAl6V4 surfaces are not invulnerable to bacterial adhesion and can be colonized by *Serratia spp*., sulphur-oxidizing and sulphate-reducing bacteria (Cwalina et al., 2017) and by clinical isolates of *Streptococcus*, *Staphylococcus* and *Escherichia coli* (Wang et al., 2018). Therefore, properties such as surface hydrophobicity and roughness alone or even combined are not the only factors that mediate bacterial adhesion and the development into a mature

biofilm. In fact, Gyo et al. (2008) coined that the relationship between hydrophobicity and biofilm formation is controversial and put forward that the adhesion process in immersed biofilms is even more complicated because of potential anomalies between surface properties and submerged conditions.

Biofilm architecture

Biofilms were also observed under the SEM. On glass, *C. metallidurans* CH34 biofilms grew either as single cells, cells clustered in a polymeric extracellular matrix or dense globular clusters (Figure 5a, b, c and d). On Teflon, the density of single adherent cells was higher than for glass, which is in agreement with CV quantification. The cell clusters were also larger than those observed on glass. In addition, Teflon-adherent clusters were held together and anchored to the surface by a complex filamentous web (Figure 5e, f, g and h). The web was composed of interconnected thin strands of 20 to 30 nm width that can reach nearly two µm in length (Figure 5g and h). Interestingly, these filamentous networks were observed neither in glass-adherent *C. metallidurans* CH34 biofilms (Figure 5a, b, c and d) nor in planktonic cells grown in the presence of Teflon and of glass (Supplementary Figure 3). Such structures were reported in biofilms of *Enterococcus faecalis* (Barnes et al., 2012), *P. aeruginosa* (Wang et al., 2015), *Ralstonia solanacearum* (Minh Tran et al., 2016) and *Streptococcus mutans* (Kim et al., 2018), and are most likely composed of extracellular DNA (eDNA). The latter is a key stabilizing element in many bacterial biofilms (Campoccia et al., 2021; Okshevsky and Meyer, 2015; Panlilio and Rice, 2021). The release of eDNA is facilitated either through cellular lysis or via membrane vesicles (Panlilio and Rice, 2021). Indeed, we observed that biofilm cells were covered with outer membrane vesicles (OMVs) of 20 to 50 nm width that putatively could release proteins or nucleic acids (Figure 5h). The resulting multilayered structure was capable of capturing solid components present in

the media such as suspended crystals or debris, reinforcing further the biofilm (Figure 5f). In addition, unique polar protrusions connecting cells in an organized manner were also observed (Supplementary Figure 4). Overall, SEM observations revealed two distinguishable adhesion patterns for *C. metallidurans* CH34, i.e. monolayers of scattered matrix-free cells and complex multilayered clusters or communities.

As previously described, glass and Teflon differ drastically in their physicochemical properties and it is possible that these dissimilarities induced a different attachment strategy. In fact, it has been shown that the presence of eDNA significantly increased the cell envelope hydrophobicity of *S. epidermidis* and allowed for a stronger preference to hydrophobic surfaces through acid-base interactions (Das et al., 2010). Likewise, for *C. metallidurans* CH34, eDNA putatively mediated attachment to the hydrophobic surface of Teflon and could be disadvantageous for the adhesion to hydrophilic glass. Many bacteria can actively regulate the release of eDNA (Ibanez de Aldecoa et al., 2017; Minh Tran et al., 2016) and we hypothesize that this is also the case for *C. metallidurans* CH34. To investigate the role of the observed net-like frameworks further, we exposed mature *C. metallidurans* CH34 biofilms on Teflon to DNaseI. This treatment apparently reduced the thickness and cell density at the edge of the cluster, suggesting that eDNA has a role in aggregation and cell build-up at the boundaries of the biofilms (Figure 6). Nevertheless, DNase I treatment did not affect the total adherent biomass quantified via CV (data not shown) and appeared to have a limited effect on the mature biofilms. In addition, it has been shown that the efficiency of DNase treatment was minimal in mature biofilms of *E. coli, Klebsiella pneumoniae*, *P. aeruginosa* and *S. mutans*, because mature biofilms accumulated more Z-form eDNA that is, unlike the B-form, resistant to DNaseI (Buzzo et al., 2021).

Elemental release from the test surfaces

Next, the possible release of elements from SS316 and TiAl64, and the role of *C. metallidurans* CH34 herein was investigated. SS316 is mainly composed of iron (at least 65%), next to chromium (16-18%), nickel (10-14%) and molybdenum (2-3%). A significant higher Fe release was observed in the media of abiotic controls at pH 7.0 compared to when SS316 was incubated with bacteria (Figure 7, bottom). Gluconate probably acted as a chelator and released Fe from the surface (Sawyer, 1964). Fe leaching from the surface in the presence of *C. metallidurans* is probably reduced as gluconate is consumed. No significant differences in Fe content of the bacterial biomass were observed in the presence or absence of SS316 (Figure 7, bottom). The TiAl6V4 alloy contains next to titanium, at least 6% aluminum and 4% vanadium. No significant differences in the aluminum levels of the cell-free supernatant of the biotic samples and abiotic controls were found at pH 5.0 and pH 7.0. Interestingly, when cells were incubated with the TiAl6V4 surface at pH 7.0 the biomass contained nearly 2 times more aluminum compared to the biotic control. This difference was not observed at pH 5.0. Overall, this indicated more release of Al from the titanium alloy at pH 7.0. Finally, elemental release from Teflon, which is considered one of the most chemically inert polymers and non-degradable by microorganisms under aerobic and anaerobic conditions, was not investigated.

Conclusions

Our study on biofilm formation of *C. metallidurans* CH34 on four space-relevant surfaces showed that stainless steel SS316 and the titanium alloy TiAl6V4 were less prone to biofouling in the tested conditions (i.e. mineral growth medium with gluconate as sole carbon source in turbulent flow conditions). The use of these materials for spaceflight applications, such as water management systems, can thus be beneficial

364 to prevent the build-up of *Cupriavidus metallidurans*biofilms . However, as the
365 contamination in such systems is composed of multiple interacting species, multispecies
366 experiments should be scrutinized in a next step. Interestingly, in the water-dispensing
367 unit designed lately by NASA, stainless steel sections in contact with water were
368 replaced by tubing in Teflon. As we demonstrated here, Teflon surfaces are highly
369 attractive for *Cupriavidus metallidurans* and the frequent dispensing function of such
370 equipment represents a potential entry point for this resilient bacterium (Maryatt, 2018).
371 It is reasonable to assume that coating and grafting of the studied materials with
372 antimicrobials may improve their performance in time as well as versus other bacterial
373 contaminants.

374 Our experiments also revealed that surface hydrophobicity and roughness alone
375 or even combined are not the only factors that drive bacterial adhesion. Despite the
376 differences in their physicochemical characteristics, both hydrophobic fluoropolymeric
377 (Teflon) and hydrophilic borosilicate (glass) material allowed extensive biofilm
378 formation via monolayers of scattered matrix-free cells and complex multilayered
379 clusters or communities. In addition, *C. metallidurans* CH34 likely employs a distinct
380 attachment machinery depending on the physicochemical characteristics of the surface.
381 We showed that filamentous structures described as extracellular DNA networks were
382 specific to the complex multilayered clusters growing on Teflon.

383 These biofilms were only locally sensitive to the enzymatic action of DNaseI,
384 suggesting that the filamentous eDNA mesh may have a specific role in CH34 biofilm's
385 architecture. As such, the use of enzyme-based antifouling products that induce the
386 hydrolysis of DNA should be carefully studied (Okshevsky et al., 2015). These products
387 are reported to weaken biofilm interactions and increase the permeability to
388 antimicrobial treatments (Okshevsky et al., 2015). For developing anti-biofilm protocols

389 it is important to take into account that bacterial adhesion is a complex phenomenon
390 where attached cells from multiple different species can be in different phenotypic and
391 metabolic states and consequently have different sensitivity to treatments.
392 Finally, it is important to remind that water recycling and recovery systems are
393 composed of numerous other materials and all are interacting indirectly through the
394 flowing liquid. Therefore, possible consequences of the observed iron leaching and
395 aluminum accumulation should be investigated in long-term experiments.
396

References

Acres, J.M., Youngapelian, M.J., Nadeau, J., 2021. The influence of spaceflight and simulated microgravity on bacterial motility and chemotaxis. NPJ Microgravity 7(1), 7. https://doi.org/10.1038/s41526-021-00135-x.

Alfa, M.J., Ribeiro, M.M., da Costa Luciano, C., Franca, R., Olson, N., DeGagne, P., Singh, H., 2017. A novel polytetrafluoroethylene-channel model, which simulates low levels of culturable bacteria in buildup biofilm after repeated endoscope reprocessing. Gastrointest. Endosc. 86(3), 442-451 e441. https://doi.org/10.1016/j.gie.2017.05.014.

Barnes, A.M., Ballering, K.S., Leibman, R.S., Wells, C.L., Dunny, G.M., 2012. *Enterococcus faecalis* produces abundant extracellular structures containing DNA in the absence of cell lysis during early biofilm formation. mBio 3(4), e00193-00112. https://doi.org/10.1128/mBio.00193-12.

Berggren, D., Bertling, S., Heijerick, D., Herting, G., Koundakjian, P., Leygraf, C., Odnevall Wallinder, I., 2004. Release of Chromium, Nickel and Iron from Stainless Steel Exposed under Atmospheric Conditions and The Environmental Interaction of these Metals : A Combined Field and Laboratory Investigation, Eurofer and Swedish Steel Association.

Bijlani, S., Stephens, E., Singh, N.K., Venkateswaran, K., Wang, C.C.C., 2021. Advances in space microbiology. iScience 24(5), 102395. https://doi.org/10.1016/j.isci.2021.102395.

Buzzo, J.R., Devaraj, A., Gloag, E.S., Jurcisek, J.A., Robledo-Avila, F., Kesler, T., Wilbanks, K., Mashburn-Warren, L., Balu, S., Wickham, J., Novotny, L.A., Stoodley, P., Bakaletz, L.O., Goodman, S.D., 2021. Z-form extracellular DNA is a structural component of the bacterial biofilm matrix. Cell 184(23), 5740-5758 e5717. https://doi.org/10.1016/j.cell.2021.10.010.

Byloos, B., Coninx, I., Van Hoey, O., Cockell, C., Nicholson, N., Ilyin, V., Van Houdt, R., Boon, N., Leys, N., 2017. The Impact of Space Flight on Survival and Interaction of *Cupriavidus metallidurans* CH34 with Basalt, a Volcanic Moon Analog Rock. Front. Microbiol. 8, 671. https://doi.org/10.3389/fmicb.2017.00671.

Campoccia, D., Montanaro, L., Arciola, C.R., 2021. Extracellular DNA (eDNA). A Major Ubiquitous Element of the Bacterial Biofilm Architecture. Int. J. Mol. Sci. 22(16). https://doi.org/10.3390/ijms22169100.

Cerca, N., Pier, G.B., Vilanova, M., Oliveira, R., Azeredo, J., 2005. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of *Staphylococcus epidermidis*. Res. Microbiol. 156(4), 506-514. https://doi.org/10.1016/j.resmic.2005.01.007.

Cockell, C.S., Santomartino, R., Finster, K., Waajen, A.C., Eades, L.J., Moeller, R., Rettberg, P., Fuchs, F.M., Van Houdt, R., Leys, N., Coninx, I., Hatton, J., Parmitano, L., Krause, J., Koehler, A., Caplin, N., Zuijderduijn, L., Mariani, A., Pellari, S.S., Carubia, F., Luciani, G., Balsamo, M., Zolesi, V., Nicholson, N., Loudon, C.M., Doswald-Winkler, J., Herova, M., Rattenbacher, B., Wadsworth, J., Craig Everroad, R., Demets, R., 2020. Space station biomining experiment demonstrates rare earth element extraction in microgravity and Mars gravity. Nat Commun 11(1), 5523. https://doi.org/10.1038/s41467-020-19276-w.

Cwalina, B., Dec, W., Michalska, J.K., Jaworska-Kik, M., Student, S., 2017. Initial stage of the biofilm formation on the NiTi and Ti6Al4V surface by the sulphur-oxidizing bacteria and sulphate-reducing bacteria. J. Mater. Sci. Mater. Med. 28(11), 173. https://doi.org/10.1007/s10856-017-5988-2.

Das, T., Sharma, P.K., Busscher, H.J., van der Mei, H.C., Krom, B.P., 2010. Role of extracellular DNA in initial bacterial adhesion and surface aggregation. Appl. Environ. Microbiol. 76(10), 3405-3408. https://doi.org/10.1128/AEM.03119-09.
De Gelder, J., Vandenabeele, P., De Boever, P., Mergeay, M., Moens, L., De Vos, P., 2009. Raman Spectroscopic Analysis of Cupriavidus metallidurans LMG 1195 (CH34) Cultured in Low-shear Microgravity Conditions. Microgravity Science and Technology 21(3), 217-223. https://doi.org/10.1007/s12217-008-9037-0.
Diaz, A., Li, W., Irwin, T.D., Calle, L.M., Callahan, M.R., 2019. Investigation of Biofilm Formation and Control for Spacecraft - An Early Literature Review, 49th International Conference on Environmental Systems. Boston, Massachusetts.
Flemming, H.C., 1998. Relevance of biofilms for the biodeterioration of surfaces of polymeric materials. Polym. Degradation Stab. 59(1-3), 309-315. https://doi.org/Doi 10.1016/S0141-3910(97)00189-4.
Guan, N., Liu, L., 2020. Microbial response to acid stress: mechanisms and applications. Appl Microbiol Biotechnol 104(1), 51-65. https://doi.org/10.1007/s00253-019-10226-1.
Gyo, M., Nikaido, T., Okada, K., Yamauchi, J., Tagami, J., Matin, K., 2008. Surface response of fluorine polymer-incorporated resin composites to cariogenic biofilm adherence. Appl. Environ. Microbiol. 74(5), 1428-1435. https://doi.org/10.1128/AEM.02039-07.
Gyurcsik, B., Nagy, L., 2000. Carbohydrates as ligands: coordination equilibria and structure of the metal complexes. Coord. Chem. Rev. 203(1), 81-149. https://doi.org/https://doi.org/10.1016/S0010-8545(99)00183-6.
Hamadi, F., Latrache, H., Zekraoui, M., Ellouali, M., Bengourram, J., 2009. Effect of pH on surface energy of glass and Teflon and theoretical prediction of *Staphylococcus aureus* adhesion. Materials Science & Engineering C-Biomimetic and Supramolecular Systems 29(4), 1302-1305. https://doi.org/10.1016/j.msec.2008.10.023.
Harrison, J.J., Ceri, H., Turner, R.J., 2007. Multimetal resistance and tolerance in microbial biofilms. Nat. Rev. Microbiol. 5(12), 928-938. https://doi.org/10.1038/nrmicro1774.
Hedberg, Y.S., Odnevall Wallinder, I., 2015. Metal release from stainless steel in biological environments: A review. Biointerphases 11(1), 018901. https://doi.org/10.1116/1.4934628.
Herting, G., Wallinder, I.O., Leygraf, C., 2006. Factors that influence the release of metals from stainless steels exposed to physiological media. Corros. Sci. 48(8), 2120-2132. https://doi.org/10.1016/j.corsci.2005.08.006.
Horneck, G., Klaus, D.M., Mancinelli, R.L., 2010. Space microbiology. Microbiol Mol Biol Rev 74(1), 121-156. https://doi.org/10.1128/mmbr.00016-09.
Huang, B., Li, D.G., Huang, Y., Liu, C.T., 2018. Effects of spaceflight and simulated microgravity on microbial growth and secondary metabolism. Mil Med Res 5(1), 18. https://doi.org/10.1186/s40779-018-0162-9.
Ibanez de Aldecoa, A.L., Zafra, O., Gonzalez-Pastor, J.E., 2017. Mechanisms and Regulation of Extracellular DNA Release and Its Biological Roles in Microbial Communities. Front. Microbiol. 8, 1390. https://doi.org/10.3389/fmicb.2017.01390.
Ishida, Y., Kadota, H., 1981. Growth patterns and substrate requirements of naturally occurring obligate oligotrophs. Microb. Ecol. 7(2), 123-130. https://doi.org/10.1007/BF02032494.
Jacobsen, M., Clausen, P.P., Smidth, S., 1980. The effect of fixation and trypsinization on the immunohistochemical demonstration of intracellular immunoglobulin in paraffin

embedded material. Acta Pathol. Microbiol. Scand. A 88(6), 369-376. https://doi.org/10.1111/j.1699-0463.1980.tb02508.x.
Kim, M., Jeon, J., Kim, J., 2018. *Streptococcus mutans* extracellular DNA levels depend on the number of bacteria in a biofilm. Sci. Rep. 8(1), 13313. https://doi.org/10.1038/s41598-018-31275-y.
Klintworth, R., Reher, H.J., Viktorov, A.N., Bohle, D., 1999. Biological induced corrosion of materials II: new test methods and experiences from MIR station. Acta Astronaut. 44(7-12), 569-578.
Leroy, B., Rosier, C., Erculisse, V., Leys, N., Mergeay, M., Wattiez, R., 2010. Differential proteomic analysis using isotope-coded protein-labeling strategies: comparison, improvements and application to simulated microgravity effect on Cupriavidus metallidurans CH34. Proteomics 10(12), 2281-2291. https://doi.org/10.1002/pmic.200900286.
Leys, N., Baatout, S., Rosier, C., Dams, A., s'Heeren, C., Wattiez, R., Mergeay, M., 2009. The response of *Cupriavidus metallidurans* CH34 to spaceflight in the international space station. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 96(2), 227-245. https://doi.org/10.1007/s10482-009-9360-5.
Liu, J., Madec, J.Y., Bousquet-Melou, A., Haenni, M., Ferran, A.A., 2021. Destruction of *Staphylococcus aureus* biofilms by combining an antibiotic with subtilisin A or calcium gluconate. Sci. Rep. 11(1), 6225. https://doi.org/10.1038/s41598-021-85722-4.
Luo, T.L., Hayashi, M., Zsiska, M., Circello, B., Eisenberg, M., Gonzalez-Cabezas, C., Foxman, B., Marrs, C.F., Rickard, A.H., 2019. Introducing BAIT (Biofilm Architecture Inference Tool): a software program to evaluate the architecture of oral multi-species biofilms. Microbiology (Reading) 165(5), 527-537. https://doi.org/10.1099/mic.0.000761.
Maertens, L., Coninx, I., Claesen, J., Leys, N., Matroule, J.Y., Van Houdt, R., 2020. Copper Resistance Mediates Long-Term Survival of Cupriavidus metallidurans in Wet Contact With Metallic Copper. Front. Microbiol. 11, 1208. https://doi.org/10.3389/fmicb.2020.01208.
Mah, T.F., O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 9(1), 34-39. https://doi.org/10.1016/s0966-842x(00)01913-2.
Maryatt, B.W., 2018. Lessons learned for the International Space Station Potable Water Dispenser, 48th International Conference on Environmental Systems. Albuquerque, New Mexico.
Mergeay, M., Nies, D., Schlegel, H.G., Gerits, J., Charles, P., Van Gijsegem, F., 1985. *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. J. Bacteriol. 162(1), 328-334.
Mijnendonckx, K., Ali, M.M., Provoost, A., Janssen, P., Mergeay, M., Leys, N., Charlier, D., Monsieurs, P., Van Houdt, R., 2019. Spontaneous mutation in the AgrRS two-component regulatory system of *Cupriavidus metallidurans* results in enhanced silver resistance. Metallomics 11(11), 1912-1924. https://doi.org/10.1039/c9mt00123a.
Mijnendonckx, K., Provoost, A., Ott, C.M., Venkateswaran, K., Mahillon, J., Leys, N., Van Houdt, R., 2013. Characterization of the Survival Ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from Space-Related Environments. Microb. Ecol. 65(2), 347-360. https://doi.org/10.1007/s00248-012-0139-2.
Minh Tran, T., MacIntyre, A., Khokhani, D., Hawes, M., Allen, C., 2016. Extracellular DNases of *Ralstonia solanacearum* modulate biofilms and facilitate bacterial wilt virulence. Environ. Microbiol. 18(11), 4103-4117. https://doi.org/10.1111/1462-2920.13446.

Mumme, T., Müller-Rath, R., Jakobi, N., Weißkopf, M., Dott, W., Marx, R., Wirtz, D.-C., 2005. In vitro serum levels of metal ions released from orthopaedic implants. Eur. J. Orthop. Surg. Traumatol. 15(2), 83-89. https://doi.org/10.1007/s00590-004-0206-6.

National Research Council, 2000. Methods for Developing Spacecraft Water Exposure Guidelines. The National Academies Press, Washington, DC. https://doi.org/doi:10.17226/9892.

Okshevsky, M., Meyer, R.L., 2015. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. Crit. Rev. Microbiol. 41(3), 341-352. https://doi.org/10.3109/1040841X.2013.841639.

Okshevsky, M., Regina, V.R., Meyer, R.L., 2015. Extracellular DNA as a target for biofilm control. Curr. Opin. Biotechnol. 33, 73-80. https://doi.org/10.1016/j.copbio.2014.12.002.

Padan, E., Bibi, E., Ito, M., Krulwich, T.A., 2005. Alkaline pH homeostasis in bacteria: new insights. Biochim Biophys Acta 1717(2), 67-88. https://doi.org/10.1016/j.bbamem.2005.09.010.

Pal, A., Paul, A.K., 2013. Optimization of Cultural Conditions for Production of Extracellular Polymeric Substances (EPS) by Serpentine Rhizobacterium *Cupriavidus pauculus* KPS 201. Journal of Polymers 2013, 692374. https://doi.org/10.1155/2013/692374.

Panlilio, H., Rice, C.V., 2021. The role of extracellular DNA in the formation, architecture, stability, and treatment of bacterial biofilms. Biotechnol Bioeng 118(6), 2129-2141. https://doi.org/10.1002/bit.27760.

Percival, S., 1999. The effect of molybdenum on biofilm development. J Ind Microbiol Biotechnol 23(2), 112-117. https://doi.org/10.1038/sj.jim.2900712.

Percival, S.L., Beech, I.B., Edyvean, R.G.J., Knapp, J.S., Wales, D.S., 1997. Biofilm development on 304 and 316 stainless steels in a potable water system. Water and Environmental Journal 11(4), 289-294. https://doi.org/https://doi.org/10.1111/j.1747-6593.1997.tb00131.x.

Petala, M., Tsiridis, V., Darakas, E., Kostoglou, M., 2020. Longevity Aspects of Potable Water Disinfected by Ionic Silver: Kinetic Experiments and Modeling. Water 12(1), 258. https://doi.org/ARTN 258
10.3390/w12010258.

Roman, M.C., Minton-Summers, S., 1998. Assessment of biofilm formation in the International Space Station Water Recovery and Management system. Life Support Biosph. Sci. 5(1), 45-51.

Santomartino, R., Waajen, A.C., de Wit, W., Nicholson, N., Parmitano, L., Loudon, C.M., Moeller, R., Rettberg, P., Fuchs, F.M., Van Houdt, R., Finster, K., Coninx, I., Krause, J., Koehler, A., Caplin, N., Zuijderduijn, L., Zolesi, V., Balsamo, M., Mariani, A., Pellari, S.S., Carubia, F., Luciani, G., Leys, N., Doswald-Winkler, J., Herova, M., Wadsworth, J., Everroad, R.C., Rattenbacher, B., Demets, R., Cockell, C.S., 2020. No Effect of Microgravity and Simulated Mars Gravity on Final Bacterial Cell Concentrations on the International Space Station: Applications to Space Bioproduction. Front. Microbiol. 11, 579156. https://doi.org/10.3389/fmicb.2020.579156.

Sawyer, D.T., 1964. Metal-Gluconate Complexes. Chem. Rev. 64(6), 633-643. https://doi.org/10.1021/cr60232a003.

Schultz, J.R., Taylor, R.D., Flanagan, D.T., Carr, S.E., Bruce, R.J., Svoboda, J.V., Huls, M.H., Sauer, R.L., Pierson, D.L., 1991. Biofilm Formation and Control in a Simulated Spacecraft Water System: Two-Year Results. SAE Transactions 100, 1056-1066.

Shelobolina, E.S., Walker, D.K., Parker, A.E., Lust, D.V., Schultz, J.M., Dickerman, G.E., 2018. Inactivation of *Pseudomonas aeruginosa* biofilms formed under high shear stress on various hydrophilic and hydrophobic surfaces by a continuous flow of ozonated water. Biofouling 34(7), 826-834. https://doi.org/10.1080/08927014.2018.1506023.
Sheng, X., Ting, Y.P., Pehkonen, S.O., 2008. The influence of ionic strength, nutrients and pH on bacterial adhesion to metals. J Colloid Interface Sci 321(2), 256-264. https://doi.org/10.1016/j.jcis.2008.02.038.
Siems, K., Müller, D.W., Maertens, L., Ahmed, A., Van Houdt, R., Mancinelli, R.L., Baur, S., Brix, K., Kautenburger, R., Caplin, N., Krause, J., Demets, R., Vukich, M., Tortora, A., Roesch, C., Holland, G., Laue, M., Mücklich, F., Moeller, R., 2022. Testing Laser-Structured Antimicrobial Surfaces Under Space Conditions: The Design of the ISS Experiment BIOFILMS. Frontiers in Space Technologies 2, 773244. https://doi.org/10.3389/frspt.2021.773244.
Sinde, E., Carballo, J., 2000. Attachment of *Salmonella* spp. and *Listeria monocytogenes* to stainless steel, rubber and polytetrafluorethylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol. 17(4), 439-447. https://doi.org/DOI 10.1006/fmic.2000.0339.
Squire, M.D., Rotter, H.A., Lee, J., Packham, N., Brady, T.K., Kelly, R., Ott, C.M., 2014. International Space Station (ISS) Orbital Replaceable Unit (ORU) Wet Storage Risk Assessment. NASA.
Stoodley, P., Sauer, K., Davies, D.G., Costerton, J.W., 2002. Biofilms as complex differentiated communities. Annu. Rev. Microbiol. 56(1), 187-209. https://doi.org/10.1146/annurev.micro.56.012302.160705.
Thompson, A.F., English, E.L., Nock, A.M., Willsey, G.G., Eckstrom, K., Cairns, B., Bavelock, M., Tighe, S.W., Foote, A., Shulman, H., Pericleous, A., Gupta, S., Kadouri, D.E., Wargo, M.J., 2020. Characterizing species interactions that contribute to biofilm formation in a multispecies model of a potable water bacterial community. Microbiology (Reading) 166(1), 34-43. https://doi.org/10.1099/mic.0.000849.
Van Houdt, R., Leys, N., 2020. Monitoring the Microbial Burden in Manned Space Stations, in: Choukèr, A. (Ed.) Stress Challenges and Immunity in Space: From Mechanisms to Monitoring and Preventive Strategies. Springer International Publishing, Cham, pp. 463-475. https://doi.org/10.1007/978-3-030-16996-1_25.
Van Houdt, R., Michiels, C.W., 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. J. Appl. Microbiol. 109(4), 1117-1131. https://doi.org/10.1111/j.1365-2672.2010.04756.x.
Van Houdt, R., Vandecraen, J., Leys, N., Monsieurs, P., Aertsen, A., 2021. Adaptation of *Cupriavidus metallidurans* CH34 to Toxic Zinc Concentrations Involves an Uncharacterized ABC-Type Transporter. Microorganisms 9(2), 309. https://doi.org/10.3390/microorganisms9020309.
Wang, A., Jones, I.P., Landini, G., Mei, J., Tse, Y.Y., Li, Y.X., Ke, L., Huang, Y., Liu, L.I., Wang, C., Sammons, R.L., 2018. Backscattered electron imaging and electron backscattered diffraction in the study of bacterial attachment to titanium alloy structure. J Microsc 270(1), 53-63. https://doi.org/10.1111/jmi.12649.
Wang, D., Jia, R., Kumseranee, S., Punpruk, S., Gu, T.Y., 2021. Comparison of 304 and 316 stainless steel microbiologically influenced corrosion by an anaerobic oilfield biofilm consortium. Eng. Failure Anal. 122, 105275. https://doi.org/ARTN 105275 10.1016/j.engfailanal.2021.105275.
Wang, S., Liu, X., Liu, H., Zhang, L., Guo, Y., Yu, S., Wozniak, D.J., Ma, L.Z., 2015. The exopolysaccharide Psl-eDNA interaction enables the formation of a biofilm

skeleton in *Pseudomonas aeruginosa*. Environ. Microbiol. Rep. 7(2), 330-340. https://doi.org/10.1111/1758-2229.12252.
Wieland, P.O., Center, G.C.M.S.F., 1998. Living Together in Space: The Design and Operation of the Life Support Systems on the International Space Station. National Aeronautics and Space Administration, Marshall Space Flight Center.
Yang, J., Barrila, J., Mark Ott, C., King, O., Bruce, R., McLean, R.J.C., Nickerson, C.A., 2021. Longitudinal characterization of multispecies microbial populations recovered from spaceflight potable water. NPJ Biofilms Microbiomes 7(1), 70. https://doi.org/10.1038/s41522-021-00240-5.
Zea, L., McLean, R.J.C., Rook, T.A., Angle, G., Carter, D.L., Delegard, A., Denvir, A., Gerlach, R., Gorti, S., McIlwaine, D., Nur, M., Peyton, B.M., Stewart, P.S., Sturman, P., Velez Justiniano, Y.A., 2020. Potential biofilm control strategies for extended spaceflight missions. Biofilm 2. https://doi.org/10.1016/j.bioflm.2020.100026.
Zhang, M., Chen, S., Gnegy, M., Ye, C., Lin, W., Lin, H., Yu, X., 2018. Environmental strains potentially contribute to the proliferation and maintenance of antibiotic resistance in drinking water: A case study of Cupriavidus metallidurans. Sci. Total Environ. 643, 819-826. https://doi.org/10.1016/j.scitotenv.2018.06.013.
Zheng, S., Bawazir, M., Dhall, A., Kim, H.E., He, L., Heo, J., Hwang, G., 2021. Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. Front Bioeng Biotechnol 9, 643722. https://doi.org/10.3389/fbioe.2021.643722.
Zhou, S., Schoneich, C., Singh, S.K., 2011. Biologics formulation factors affecting metal leachables from stainless steel. AAPS PharmSciTech 12(1), 411-421. https://doi.org/10.1208/s12249-011-9592-3.

669 Figure 1. SEM images of the test materials, i.e. glass (a), stainless steel SS316 (b),
670 Teflon (c) and titanium alloy TiAl6V4 (d).

671 Figure 2: Contact angle measurements of test materials in MM284 at pH 5.0 (red) and
672 pH 7.0 (blue), and water (green). The average values of three independent experiments
673 (n=6) with standard deviations are shown. Brackets indicate which samples within the
674 surface set are statistically different ($p < 0.05$) based on ANOVA and post-hoc Tukey.

675 Figure 3: Growth of *C. metallidurans* CH34 in the absence (black) or presence of glass
676 (red), stainless steel SS316 (blue), titanium alloy TiAl6V4 (green) or Teflon (purple)
677 surface. The average values of three independent experiments with standard deviations
678 are shown.

679 Figure 4. Crystal violet quantification of *C. metallidurans* CH34 biofilm formation after
680 168 hours at pH 5.0 (red) and 7.0 (blue) on the test materials. The average values of
681 three independent experiments with standard deviations are shown. For each pH value,
682 the different letters above the error bars indicate significant differences ($p < 0.05$) based
683 on ANOVA and post-hoc Tukey.

684 Figure 5. Representative SEM images of *C. metallidurans* CH34 biofilms on glass (a, b,
685 c and d) and Teflon (e, f, g and h).

686 Figure 6. Effect of DNAseI treatment (b) versus control (a) on Teflon-grown
687 *C. metallidurans* biofilms visualized by crystal violet staining.

688 Figure 7. ICP-MS analysis of Al release from titanium alloy TiAl6V4 (top panel) and
689 Fe from stainless steel SS316 (bottom panel) by *C. metallidurans* CH34 (left) and
690 uninoculated growth medium (middle), and compared with their content in
691 *C. metallidurans* cells (right) for both the liquid (light grey) and bacterial cell (dark
692 grey) fraction. The average values of three independent experiments (n=3) with
693 standard deviations are shown.

694 Supplementary Figure 1: Crystal violet staining illustrating biofilm formation by *C.*
695 *metallidurans* CH34 on glass (left panel) and Teflon (right panel) after 168h in pH 5.0.

696 Supplementary Figure 2: Crystal violet quantification (dark grey) and plate count (light
697 grey) of *C. metallidurans* CH34 biofilm formation after 168 hours at pH 5.0 on glass

and Teflon. The average values of three independent experiments with standard deviations are shown. The different letters above the error bars indicate significant differences ($p < 0.05$) based on ANOVA and post-hoc Tukey.

Supplementary Figure 2: (a) Planktonic cells of *C.metallidurans* CH34 cultured in the presence of Teflon versus (b) planktonic cells cultured in the presence of glass.

Supplementary Figure 3: Polar protrusions and linear organisation of *C.metallidurans* CH34 cells adherent to Teflon.