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# Effect of extracellular polymeric substances on the mechanical properties of *Rhodococcus*

Yu Pen [a,b], Zhenyu J. Zhang [a,*], Ana L. Morales-García [a], Matthew Mears [a], Drew S. Tarmey [c], Robert G. Edyvean [b], Steven A. Banwart [d], Mark Geoghegan [a]

[a] Department of Physics and Astronomy, University of Sheffield, Sheffield S3 7RH, UK
[b] Department of Chemical and Biological Engineering, University of Sheffield, Sheffield S1 3JD, UK
[c] School of Medicine, University of Nottingham, Royal Derby Hospital, Uttoxeter Road, Derby DE22 3DT, UK
[d] Department of Civil and Structural Engineering, University of Sheffield, Sheffield S3 7HQ, UK

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ABSTRACT

The mechanical properties of *Rhodococcus* RC291 were measured using force spectroscopy equipped with a bacterial cell probe. Rhodococcal cells in the late growth stage of development were found to have greater adhesion to a silicon oxide surface than those in the early growth stage. This is because there are more extracellular polymeric substances (EPS) that contain nonspecific binding sites available on the cells of late growth stage. It is found that EPS in the late exponential phase are less densely bound but consist of chains able to extend further into their local environment, while the denser EPS at the late stationary phase act more to sheath the cell. Contraction and extension of the EPS could change the density of the binding sites, and therefore affect the magnitude of the adhesion force between the EPS and the silicon oxide surface. By treating rhodococcal EPS as a surface-grafted polyelectrolyte layer and using scaling theory, the interaction between EPS and a solid substrate was modelled for the cell approaching the surface which revealed that EPS possess a large capacity to store charge. Changing the pH of the surrounding medium acts to change the conformation of EPS chains.



## 1. Introduction

Oil spills and toxic compounds discharged from industrial activities and agriculture are examples of processes causing significant hydrocarbon contamination. Bioremediation is regarded as a non-destructive, cost-effective and environmentally friendly way to clean up the pollutants under circumstances which limit the viability of other remediation strategies [1]. Several members of the genus *Rhodococcus* have been widely used in bioremediation due to their ability to adapt to temperature [2,3], heavily contaminated water and soil [4,5], and radioactive environments [6]. *Rhodococci* are used to degrade xenobiotic contaminants [7], to desulphurise coal derivatives in water [8], and are involved in many engineered and in situ bioremediation processes to reduce contaminant loads in water and soil [7,9]. In addition, most *Rhodococcus* species exhibit low pathogenicity, are not eco-toxic [10], and are unlikely to generate toxins or antimicrobial compounds [11,12], indicating the utility of *Rhodococcus* species for biodegradation [13].

Extracellular polymeric substances (EPS) are crucial for cell–cell adhesion and comprise exopolysaccharides, extracellular proteins, humic substances, nucleic acids, and phospholipids [14]. There is evidence suggesting that EPS could form the framework of the biofilm matrix, determine the physicochemical properties of the biofilm [14], trap dissolved organic matter in the vicinity of the cell surface [15,16], and reduce the shear stress from water drag [17]. The physical and chemical properties of EPS at different growth stages are particularly important because the EPS determine the ability of the bacterium to trap charged contaminant colloids, adhere to substrata, and resist external forces, all of which influence the degree of bioremediation.

Efforts have been made to understand the interaction between cells and mineral surfaces, which have confirmed that the behaviour of attached cells is mediated by the physical and chemical interactions of the macromolecules at the interface [18–20]. In the present study the mechanical properties of *Rhodococcus* are modelled by considering an EPS enclosed rhodococcal cell as an analogue to a sphere with a hydrophobic core (mycolic acid covered cell wall) and a charged corona (EPS). EPS are here considered as polymers tethered to the cell surface, so that they can be treated as surface-grafted polymer chains (known as brushes, where the distance between grafting sites is shorter than the unstretched polymer end-to-end distance [21–25]). Most bacterial EPS exhibit anionic characteristics due to uronic acids (containing carboxyl

* Corresponding author at: Department of Chemical and Process Engineering, University of Strathclyde, Glasgow G1 1XJ, UK. Tel.: +44 141 5482393; fax: +44 141 5482539.
*E-mail address:* zhenyu.zhang@strath.ac.uk (Z.J. Zhang).

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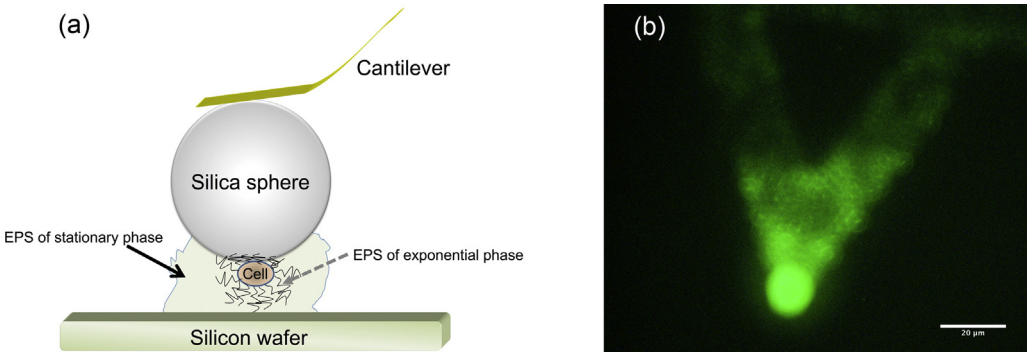


**Fig. 1.** (a) Schematic diagram of a bacterial cell probe enclosed by EPS. Cells are linked to the silica sphere via adhesive layer, but not attached to the model substrate (silicon wafer). The diagram represents an idealized view, and those cells not involved in force measurements are not shown here. (b) Fluorescence microscopy image of a cell probe that had been treated with SYTO 9 cell staining. An enhanced green colouration on the colloidal probe confirms that the particle is fully covered by rhodococcal cells.

groups), which are a major component of the exopolysaccharides [26]. It is therefore reasonable to treat EPS as weak polyelectrolytes, i.e. long chain macromolecules possessing ionizable groups [27]. This allows quantitative modelling of the repulsive force between the EPS and a solid surface when they approach each other, taking into account both electrostatic and steric forces [28]. It is worth noting that the above approach has been applied successfully not only to polyelectrolyte brushes [29,30] but also to dense layers of physisorbed homopolymers [31,32].

Initial studies of cell–surface interactions focused on the chemical structure, biological functions, and physicochemical properties of extractable EPS [33–35]. Nevertheless, measurements of interactions between extracted EPS and mineral surfaces generally ignored the contribution from non-extractable EPS and other minor components on the cell surface. The extraction process may also damage the cells, leading to the release of intracellular material and furthermore it may rupture the bonds between macromolecules, which would deform the structural integrity of the EPS. Unlike experiments using only extracted EPS, a bacterial cell probe as shown in Fig. 1 offers the opportunity to measure cell–mineral interactions under biological conditions. Several studies have been carried out to investigate steric interactions, adhesion, and viscoelasticity of the EPS of different bacterial strains [24, 36–40], but few have examined the contribution of EPS to the mechanical properties of whole cell. Bacterial cell probes made of cells chosen at different growth stages enable the characterization of the mechanical properties of *Rhodococcus* as a function of growth stage, which are important parameters in understanding its effectiveness in bioremediation.

Atomic force microscopy (AFM) can be used to measure forces between a sample surface and a fabricated probe (tip) attached to the apex of a cantilever, and measurements can be performed in liquids analogous to the natural biological environment. Force spectroscopy, a variant of AFM, measures the interaction as a function of probe-sample distance in the normal direction [41]. By attaching cells from different growth stages to the AFM cantilever, the mechanical properties of *Rhodococcus* are studied in terms of their: (1) charge storage, (2) water retention capability, and (3) adhesion to a model surface.

## 2. Materials and methods

### 2.1. Cultivation of Rhodococcus

*Rhodococcus* RC291, isolated from a contaminated gas-works site in the North East England [42], was kindly donated by J. A. C. Archer (University of Cambridge). Rhodococcal cells from a −70 °C glycerol stored stock were spread on a Petri dish containing sterile solid Luria-Bertani (LB) agar (Fisher Scientific) using a sterile loop (1 mL). Thereafter, the Petri dish was kept in an incubator at 25 °C (TSE 33644, Sanyo) for 36 h and then refrigerated at 4 °C ready for use. A liquid LB medium (Fisher Scientific) (20 g/L) was prepared using de-ionized (DI) water (Elga PURElab option, 18 MΩ·cm) prior to being sterilized in an autoclave at 121 °C for 20 min. Glucose solution (2 mM) was autoclaved separately.

A colony of *Rhodococcus* sp. RC291 was taken from the solid agar medium using a sterile loop (1 mL) and then inoculated into the LB liquid medium (100 mL) together with glucose (2 mM) in a pre-sterilized flask (300 mL). The flask was then placed on an orbital rotary shaker (DOS-20 L, ELMI Ltd.) at 150 rpm in an incubator at 25 °C for 6 h (late exponential phase), 24 h (mid-stationary phase), or 36 h (late stationary phase) based upon the growth curve shown in Fig. 2. The cell concentration was expressed in terms of optical density measured by spectrophotometry (S2100 UV/Vis Diodo Array Spectrophotometer, Biochrom, Biowave WPA).

### 2.2. Preparation of bacterial cell probe

1 mL of rhodococcal cells in LB liquid medium were transferred to a centrifuge tube (1 mL), and then centrifuged at 12,100 $g_n$ (where $g_n$ is the standard acceleration due to gravity) for 2 min, as suggested by previous studies [43–45]. (At this acceleration, only weakly bound EPS are removed as described below.) The presence of a cell pellet was confirmed after centrifugation. 0.95 mL of supernatant was removed and replaced by phosphate buffered saline (PBS: 0.2 g/L KCl, 8 g/L NaCl, 1.44 g/L $Na_2HPO_4$, and 0.24 g/L $KH_2PO_4$ at pH 7.4) [46], and vortexed to mix [47]. The PBS washing procedure was repeated three times to wash away cell debris and liquid medium remaining on the cell surfaces to enhance cell attachment onto the AFM cantilever. Cells were washed three more times using DI water prior to being concentrated. Fluorescence microscopy was used to confirm that the preparation procedure does not damage the cells.

To prepare a bacterial cell probe, a 10 µm diameter silica sphere (Duke Scientific, USA) was attached to the apex of an AFM cantilever (MLCT, Bruker probes) using epoxy glue. The colloidal probe was then immersed in 5 wt% poly(D-lysine) (weight average molecular mass 4000 g/mol) solution for 15 s to functionalize, and then exposed in air for 15 min. Poly(D-lysine) is a polycation used to bind a negatively charged cell surface through its amino groups [48,49]. The poly(D-lysine) functionalized colloidal probe was immersed in a drop of the concentrated cell solution (5 µL, ~$10^9$ cell/mL), and both were then exposed in air for 40 min. Thereafter, the probe was rinsed with DI water to remove unattached cells. The comparable sizes of a silica particle (10 µm in diameter) and a rhodococcal cell (of length ~2 µm) ensure that only one cell is measured during the experiment. A schematic diagram of an idealized bacterial cell probe enclosed by EPS is shown in Fig. 1a.

Cell staining was necessary to verify the existence of cells on the colloidal probe, and more importantly, whether the cells remain viable/active. Control experiments, applying a green-fluorescent nucleic acid