1 Introduction

The world at a microscopic level is teeming with more life than many can imagine, in fact, it is estimated that bacteria alone make up two thirds of the planet's biodiversity and 13% of all life by weight\cite{1]. Furthermore, Bacteria was among the first cellular life to exist on earth and is, to this day, found everywhere from our food to our water and bodies. For this reason, it is imperative that scientists have a comprehensive understanding of how these microscopic organisms grow and survive. Outside of an oceanic environment, Bacteria are often found in large groups, these groups tend to form communities at surfaces in what are now known as Biofilms\cite{2}. In this report, we evaluate the behaviour of microswimmers with an emphasis on their interactions at surfaces and under confinement. The remainder of the report is divided into 6 components beginning with introductory reviews of microswimmers, how they are modelled in the lab and how they move before moving into more specific properties such as motion under confinement and accumulation at surfaces. The final chapters introduce and explore biofilms before moving on to some applications of this research.

2 Properties of Microswimmers

2.1 Microswimmers in the Real World

Bacteria come in many forms and with each form are associated properties namely their motion in fluid. Broadly speaking there exist three shapes of bacteria, Coccus being spherical bacteria, Bacillus as rod-shaped bacteria and Spiral, or twisted bacteria. Within these broad groups there exist further variations of their physical structure largely pertaining to how the bacteria replicate.

Within the group of Cocci there exist:

- Diplococci from Cocci that remain in pairs after dividing
- Streptococci which emerge when Cocci remain in chains
- Tetrads formed when Cocci arrange into groups of 4 when the cells divide in two planes
- Sarcinae from Cocci that divide in three planes but remain in cubed groups of eight
- Staphylococci which are formed when Cocci form grape like structures due to dividing in multiple planes

Within the Bacilli Group:

- Diplobacilli which, as above, emerge when individual Bacilli remain in pairs after diving
- Streptobacilli emerge when the division of the Bacilli leads to the formation of an end to end chain
- Coccobacilli are a form of Bacilli that are short and thick such that they resemble the Cocci group however remain distinct

Finally, within the Spiral group:
- **Vibrio** which are curved like in appearance
- **Spirillum** which have a helical shape but rigid bodies
- **Spirochetes** which, as for the Spirillum, have a helical shape but, their bodies are flexible

To summarize the differences in the biological swimmers, figure 1 contains a graphical depiction of each as well as an SEM image for reference.

![Figure 1: Types of microswimmers and their physical combinations][3]

In liquids, these microswimmers have evolved methods by which they can move around and explore their environment, this takes place in two different ways. Self propelled or Brownian motion, wherein the bacteria will use a biological process to move through the water, and hydrodynamic interactions, which are responses of the fluid around the swimmer to its motion, both of which can be studied to develop models of the swimmer and make predictions.

To understand the biological self-propulsion, three systems will be discussed, motion of flagellated microswimmers, motion of Listeria and the motion of eukaryotic cells.

### 2.1.1 Flagellated Microswimmers

Flagellated are those that move through fluids using helical filaments as a tool for propulsion. Within the group of flagellated swimmers there exist different forms based on the location of their flagella, namely, the monotrichous bacteria, possessing only a single flagellum, lophotrichous, which have multiple flagella located in a particular place, amphitrichous bacteria, whose flagellum grow on each end of their bodies and finally, peritrichous, which have multiple flagella covering their whole body and pointing in all directions[4, 5]. The method by which these flagella propel the bacteria originates in the cell, where the flagella is connected by a hook to a rotary motor, embedded within the cell wall. When ions such as Sodium or Hydrogen flow across the cell wall, the motor will induce rotation in the flagella, some of which are outlined below.

### 2.1.2 Run and Tumble Motion

Flagellated bacteria swim in a "run and tumble" motion, named as it is comprised of the microswimmer moving in a straight line followed by a random tumbling event, wherein the particle will change direction at random and move in this new direction. Run and tumble motion is a consequence of a bacteria’s search for optimal conditions, whether it be food or temperature. By extending a run phase,
bacteria may move towards a more preferential space, whereas if worsening of conditions are detected, more tumbling events will occur to change the direction of motion\[4\]. This process occurs differently depending on the configuration of the flagella on the bacteria.

- **E-coli and Salmonella** perform a run phase when their flagella, in a left handed orientation, begin to rotate counterclockwise and form a bundle\[6–8\]. During the tumble phase, one of the flagella will reverse its rotation to a clockwise direction, leading to the flagella breaking from the bundle and inducing a re-orientation of the bacteria. This change in rotational direction is accompanied by a polymorphic transition and a change from Left handedness to right handedness. Once the bacteria is re-oriented, the flagella will once again bundle, rotating counter-clockwise and begin another run phase\[4, 8–16\].

- **Rhizobium meliloti and Rhizobium lupini** have flagella which can only undergo limited polymorphic transitions and their motors are unidirectional\[17–19\]. In order to induce a tumbling event, these bacteria will modulate the rotational speed of their flagella to induce a tumbling event\[4, 17, 18\].

- **Uni-flagellated** bacteria perform a tumbling event by exploiting a buckling instability in the hook connecting the flagella to the cell wall\[4, 20\].

### 2.1.3 Listeria

Listeria is a bacterium which employs different swimming methods depending on its environment. Outside of the body, Listeria will swim by flagella as describe above, however, once at body temperature, Listeria will stop production of Flagellin, the protein that makes up the flagella, and begin a new type of motion. This motion involves the bacteria hijacking the actin cytoskeleton, the organelle responsible for powering cell migration\[21\], and using it to produce actin gel on the cell surface which forms a comet that pushes the bacterium through the cell\[4\].

### 2.1.4 Eukaryotic Cells

Eukaryotic cells are often propelled by motile hair-like extensions called Cilia, which consist of a bundle of microtubules all connected by proteins. The main difference between the flagella discussed previously and the cilia (sometimes referred to as eukaryotic flagella) is the length, a typical cilia may be 10\(\mu\)m long whereas a flagella may be around 50\(\mu\)m. The difference is most noticeable in their motion, where flagella beat in a sinusoidal wave, cilia operate in two distinct strokes or phases. In a power stroke, cillum is stretched out straight in one direction moving the swimmer quickly in one direction, this is followed by the recovery stroke, where the cillum bend, twist sideways and slowly retract\[4\].

![Figure 2](image.png)
2.2 Microswimmers in the Lab

In order to understand the microscopic world and the life within it, scientists are working to develop microscopic motors that can perform tasks and behave as their biological counterparts do. Provided here is just a brief overview of some methods currently employed to implement these micro scale motors both in real life and in simulations.

- **Bimetallic rods and microspheres** are synthetic swimmers made from bimetallic Pt-Au rods which are immersed in a solution of hydrogen peroxide (H₂O₂)[24–26]. Motion is instigated when the catalytic reaction 2H₂O₂ → 2H₂O + O₂ occurs at the Pt end of the rod, powering the system. It is not yet fully understood how this leads motion, it is theorized that the O₂ adsorption on the Au end of the rod leads to a surface tension gradient and thus motion[4].

- **Catalytic Janus Colloids** display motion by a reaction with the fluid they reside in[27, 28]. This reaction similarly suggests a non-equilibrium condition being induced and thus, a force being exerted on the particle[29–38].

- **Thermophoretic Janus Colloids** operate similarly to the previous examples except that they diffuse through a fluid by a temperature gradient induced by shining a laser beam onto the system[39–44].

- **Bubble jets** are artificial microswimmers that consist of a hollow tube with a functionalised surface that has been fabricated by electrodeposition. When placed into a peroxide solution, their functionalised surface begins a catalytic reaction liberating oxygen gas. By spontaneous symmetry breaking, this gas leaves the microtubel inducing motion in the swimmer[45–47].

- **Rotators** come in forms of discs[48, 49], spheres or dumbbells[50] constructed from super-paramagnetic materials such that application of a magnetic field leads to rotation[51].

- **Self-propelled Droplets** are aqueous droplets that are suspended in an oil that contains surfactants. In this solution, spontaneous chemical reactions with the surfactant such as bromination of mono-olein can occur, inducing propulsion[52].

- **Biomimetic microswimmers** are those built to directly mimic the motion of real microswimmers. One example is artificial sperm, with flagella built from magnetic colloids and attached to a red blood cell acting as the head[53].

Further to these examples, there are a number of theoretical and applied artificial swimmers, see reference [4] for a more comprehensive overview.

Such detail must be paid to the shape and movement of microswimmers as it describes how they will interact with their surroundings. As we will see, biofilm production and architecture can be largely motivated by not only the shape of a microswimmer but by the protein built structures that it produces[54]. With this in mind, there is a further interaction that must still be considered when examining surface interactions and confinement, they are the hydrodynamic interactions.

3 Hydrodynamic Interactions

A key property of microswimmers is their hydrodynamic interactions. As microswimmers are so small, they have a low Reynolds number, this leads to some interesting properties. In this section, these properties will not be discussed but rather the approach one often takes characterising these interactions as well as one of they solutions, namely, the dipole. When studying the fluid flow around a microswimmer one looks to the Navier-Stokes Equations written in equation 1

\[ \nabla \cdot v = 0 \] (1)

\[ \rho \left( \frac{\partial v}{\partial t} + (v \cdot \nabla)v \right) = \eta \nabla^2 v - \nabla p + f \] (2)
where $\rho$ is the density of the fluid, $\eta$ is the viscosity of the fluid, $v(r, t)$ is the position and time dependent fluid velocity field, $p(r, t)$ is the pressure field and $f(r, t)$ is an applied force. By substituting the Reynolds numbers $\text{Re} = \frac{\rho v_0 L}{\eta}$ and $\text{Re}_T = \frac{\rho L^2}{\eta T_0}$ one can reduce the Navier-Stokes equation to the dimensionless expression in equation 3.

$$\text{Re}_T \frac{\partial v'}{\partial t} + \text{Re}(v' \cdot \nabla)v' - \nabla p' + f'$$

where the prime denotes a dimensionless property. As mentioned above, microswimmers live at low Reynolds numbers, specifically $L \ll 1$, resulting in the terms on the right hand side of equation 3 becoming negligible and thus the Stokes equation takes form.

$$\nabla p - \eta \nabla^2 v = f$$

The Stokes equation is linear and time independent and is often solved for by time independent integration over the applied force as show in equation 5.

$$v(r) = \int H(r - r') \cdot g(r') d^3 r'$$

where $H(r)$ is the Oseen tensor. This integral can be solved by a multipole expansion and thus, has a number of solutions. An important solution to the Stokes equation is the dipole solution. When a swimmer moves in a fluid, the far-field hydrodynamics can be modelled by a force dipole. When this is analysed, two classes of these dipole swimmers can be produced, the pusher and the puller. The pusher swimmer having a motor on its back and the puller with a motor on its front. Importantly, it is noted the flow fields of each solution are equivalent but reversed in direction[4].

### 4 Oscillatory Motion of Microswimmers

An interesting consequence of hydrodynamic interactions takes place when a microswimmer is placed in a confined channel. When a swimmer is placed into some confining geometry, it will display helical and oscillatory motion[55]. An explanation of this behaviour is given by Graaf et al. [56] where higher order solutions to the hydrodynamic interactions are examined. The report found that the sinusoidal motion of the microswimmers was a direct consequence of the quadrupole moment whereas the Dipole and Octupole moments are responsible for not only translation, but the dampening of these oscillations. This was modelled by equation 6.

$$z(t) = z_0 e^{at} \cos(\omega t)$$

where the $\alpha$ exponent describes the dampening of the system. This process is different for pusher swimmers and puller swimmer, in the case of a pusher, the alpha constant is positive and thus, the oscillation becomes larger and larger, the opposite occurs for a puller swimmer where they will experience decaying oscillatory motion[56]. Figure 3 demonstrates these findings graphically.

![Figure 3: Oscillatory motion of Pusher and Puller swimmers in real space][56]
5 Accumulation at surfaces

In 1963, Lord Rothschild discovered surface accumulation of live sperm cells, interestingly, he found that dead cells did not display this behaviour[57]. Since this discovery, accumulation at surfaces has been recognised as a property of microswimmers and has been thoroughly investigated. In order to understand accumulation as surfaces, both the self-propulsion and hydrodynamic motion must be considered.

5.1 Brownian Motion

A microswimmer undergoing Brownian motion will eventually collide with a wall. When this collision occurs, there will be some orientational persistence, resulting in the trapping of the particle at the boundary. This persistence is strongly shape dependant and to further elucidate this, the case of self propelled rods shall be discussed further.

5.1.1 Self-propelled Rods

As a self-propelled rod nears a wall, the hydrodynamic interactions generated by its flow will tend to reorient it parallel to the wall. This orientation leads to trapping against the surface, however, in this close proximity, the rod has less possible orientations leading to an entropic repulsion of the rod. This process depends on the length of the rod being studied and thus, longer rods show smaller trapping times than smaller rods[58].

5.2 Hydrodynamic Interactions

Just as the random Brownian motion if a microswimmer allows it to interact with surfaces, the hydrodynamic interaction generated between the swimmer and the surface can lead to trapping. This can be understood by considering the induced velocity at a distance z from the surface given in equation 7.

\[ u_z(\theta, z) = -\frac{3P}{64\pi\eta z^2}(1 - \cos^2(\theta)) \]  

This equation, when studied for pusher swimmers, suggests that there is an attractive force on the swimmer when aligned parallel to a surface but furthermore, there exists a torque actively rotating a pusher to align parallel with an interface. This combination of force and torque leads to an increased trapping time at interfaces for pusher type swimmers. When studied for pullers, it is found that the torque acting on the swimmer leads to perpendicular alignment with the wall and thus, repulsion of the swimmer[58].

With this understanding of the types of bacteria and their behaviour at surfaces, one may now begin to study what happens where this takes place.

6 Biofilms

In nature, when not in the ocean, microswimmers are often found at surfaces in large communities known as biofilms[54]. Because of this, microswimmers have adapted to live under conditions as a community. When bacteria join a biofilm on a surface, they will secrete a matrix called exopolysaccharides or EPS which will act to increase the surface affinity of the bacteria. Further to this, the bacteria will also construct protein structures such as pili and fimbriae that will enhance their adhesion to the surface[54]. Biofilms are of the upmost important in modern scientific research, from medicine to town planning, due to their appearance in daily life. Figure 4 shows three examples where biofilms have formed in drastically different environments, all of which will lead to damage to the human body should they come into contact with the contaminants. One of the defining features of biofilms is their difference to their singular counterparts, for example, a lone E-coli bacteria may be quickly washed away, a biofilm of the same substance however requires extensive treatment to kill. These properties have led to vast research in the field and makes the topic of biofilms on of great interest.
6.1 Growth of a Biofilm

The growth of a biofilm is driven by nutrient availability and metabolism. When the bacteria within a biofilm are in a nutritious environment, they will begin to grow and eventually perform cell division leading to more cells in the biofilm. Naturally, the environment the biofilm develops in has a large impact on its growth. These environmental factors can be studied on a cellular scale where effects on individual or small groups of constituents, or a macroscopic scale where the architecture of the biofilm is considered.

6.2 Cellular Effects

The effects of flow over a biofilm have a great impact on its constituents, especially, in terms of their growth and structure. Two such properties are of great interest due to their importance in the survival of the film and the film’s impact on its surrounding, Nutrient Channels and Cheater Cells.

6.2.1 Nutrient Channels

When a biofilm forms it can become difficult for the bacteria at the centre of the film to receive enough nutrients to survive and grow. When cells begin to die, the biofilm will become weaker at these parts, a fluid flow over the film can lead to a wrinkling of the film, opening channels where nutrient rich fluid can be accessed by bacteria deeper in the film, thus leading to further growth[61][62][54].

6.2.2 Cheater Cells

Another problem, facing biofilms is the existence of so called Cheater Cells. When a biofilm forms, much of the nutrients that feed the cells contained within it come not from the surrounding fluid, but from the metabolic process the cells perform at the interface. This occurs when the bacteria on the surface digest the substrata which liberates nutrients for surrounding cells to feed on. This leads to the survival of cells which could otherwise not break down their substrates in order to feed, which is undesirable to the systems as a whole[63]. A flow environment can help to solve this public goods conundrum[54] as it will remove the liberated nutrients for all bacteria other than those directly surrounding the metabolising bacteria. This leads to the death of the mutant bacteria and overall strengthening of the biofilm[64].

6.3 Biofilm Architecture

The architecture of the biofilm can be described as that of a self-replicating liquid crystal often displaying local nematic ordering, that is to say, the constituents of the biofilms align over comparatively large distances[65]. Due to the emergence of this nematic ordering, biofilm architecture can be described by the cell-cell interactions that take place between the bacteria in the film and further by the flow environment it exists in[66]. To understand this architecture Hartmann et al. [66] performed an
experiment with the biofilm produced by Vibrio Cholerae. When these cells form a biofilm they secrete Vibrio polysaccharide or VPS as well as extracellular DNA and proteins which forms the matrix the cells survive within. These cells will experience an attractive pair potential mediated by the RbmA protein within the matrix which, in simulation, has been approximated by equation 8.

\[
U = \epsilon_0 \epsilon_1 \left( e^{-\frac{\rho^2}{\sigma^2}} + \frac{\nu}{1 + e^{\left(\frac{\rho - \lambda}{\sigma}\right)}} \right)
\]  

where \(\rho\) is the shape normalised cell-cell distance. The RbmA protein is essential to the stability of the biofilm as it contributes to both the binding of neighbouring cells and recruitment of external cells. It acts as a binding agent consisting often as a dimer protein with two binding surfaces which act to mediate pair attraction between neighbouring cells. The VPS will also assist in the cell binding, however, it was found that increased VPS levels do not cause stronger interaction, nor decrease cell spacing. The cells will also experience a repulsive force due to both the osmotic pressure from high concentrations of matrix components as well as steric interactions[66]. During the experiment, the growth and architecture of these biofilms is studied both under high flow and low to study the effect of shearing force. Furthermore, the effect of introducing mutants which fail to produce RbmA proteins(called \(\Delta RbmA\)) is observed.

- Figure 5: From left to right: Normal cells under low flow, mutant \(\Delta RbmA\) cell under low flow, Normal cells under high flow, mutant \(\Delta RbmA\) cells under high flow [66]

The results of this experiment are shown in figure 5. The results of the initial experiment demonstrate the effects of the RbmA protein on the density and ordering of the biofilm as one can see that there is clearly higher packing in the RbmA present film. The result of the \(\Delta RbmA\) mutation is far more present in the system under flow. With the protein, the biofilm generates a teardrop like shape due to the shearing of the surface layers, whereas the mutant bacteria is dissipated almost completely. This effect was found to be due to an increase in RbmA production as a response to the flow field. This increase in RbmA did however lead to a thinning of the film. This is due to the metabolic cost of increasing RbmA production during this response[66]. These result demonstrate the types of factors contributing to the architecture of biofilms with respect to their cell-cell attraction and behaviour under flow.

6.3.1 Streamers

Another interesting effect occurs in biofilm architecture in irregular geometries when experiencing flow, that is, the production of streamers. When a biofilm growing on a corner or bend experiences a shearing force due to flow the film will develop as a long filamentous structure within the fluid and no longer rigidly attached to the surface[67]. Whereas biofilms will inhibit flow by a small amount, streamers will cause a more substantial obstruction due to their presence at the centre-line of the path. This flow obstruction generates a positive feedback in which EPS production is favoured, leading to further development of the streamer and eventually, catastrophic obstruction of the channel[68]. As there are a number of bends in naturally occurring systems, it is suggested that this effect occurs in many systems found in nature[68].
6.4 Emergent Properties of Biofilms

The term biofilms architecture can be misleading, in reality, despite many architectures sharing common features, different biofilms may have strikingly different properties[65]. With the foundations of biofilms discussed, what follows is a brief summary of two interesting properties found in biofilms motivating further research as summarised by[59].

6.4.1 Gene Transfer

The close cell-cell distance and resistance to shearing force makes the biofilm environment great for transfer of organic material[69–71]. One example of this is the increase in plasmid production by Escherichia coli (E-coli) researched first by Ghigo [72]. Plasmids are a type of extrachromosomal DNA that have been linked to increased bacterial resistance to treatment. In the biofilm architecture, these plasmids may be transferred more freely, leading to an increase in bacterial cells resistant to antimicrobial agents.

6.5 Quorum Sensing

Xie et al. [73] demonstrated that cell-cell signaling within a biofilm may play a role in the detaching of the film from a surface. The study found that the genes involved in fimbrial expression of Porphyromonas gingivalis could be suppressed when the bacteria was exposed to unfavourable surfaces. It was found that when the P. gingivalis is exposed to a biofilm of streptococcus cristatis, the S.cristatis would suppress the genes responsible for fimbrial growth thus preventing biofilm production on the surface. This is an important process to study and understand as it could lead to new method of biofilm prevention as well as implementation.

7 Applications

With this understanding of biofilm development and structure, two applications of this knowledge are presented briefly to introduce some of the topic currently being discussed.

7.1 Biomineralization

Biomineralization is the process in which an inorganic material is produced by an organism[74]. The process by which this occurs is still not completely understood, however, three hypothesis exists.

- Mineralization occurring as a byproduct of metabolism. During these processes, reactions take place that lead to changes in the pH of the surroundings which will shift the equilibrium of carbonate molecules and lead to the production of CaCO$_3$[74].
- Nucleation of the carbonates take place due to ion exchange through the cell wall[74].
- Microbial EPS may lead to trapping of ions and thus concentration increase or may containing proteins that directly influence precipitation[74].

The process described above lead to the effective entombing of the bacteria under discussion. This effect can be utilized to perform repairs on materials that otherwise would need to be replaced such as concrete, cement and ash bricks[75]. This effect is induced by growing a biofilm of bacteria over the affected site and exposing the film to solution of urea. This system can then be left for a number of weeks as the calcium carbonate is precipitated and the material repaired. In some cases, it has been found that this process can not only repair the material, but lead to an overall increase in its strength[74].
7.2 Filtration

A common method of water purification involves allowing water to flow slowly over some porous media leading to the trapping of unwanted contaminants[76]. Slow sand filters work by forming a biofilm at the surface of the fine sand layer, as the waste flow over the biofilm, the bacteria within it will attach and the clean water may flow through[77]. Whilst this is understood, the effects of hydrodynamic interactions and motility of the microswimmers is a more difficult property to understand in this system.

![Figure 6: Hydrodynamic effects involved in filtration][77]

Figure 6 outlines a three step process in which particle motility can effect trapping times in these sand filters.

1. As the rod-like particles flow over the object a flux is present directed from the thick line in the image due to the change in vorticity of the fluid. This results in any bacteria beneath this line diffusing towards the obstacle present, in this case, filtration medium[77].

2. When bacteria reach region 2 in the image, they will experience hydrodynamic interactions from the top and bottom of the obstacle, resulting in clockwise circular rotation at the surface. It is this interaction that is suggested to lead to the trapping of the particle[77].

3. As the bacteria crawls along the surface of the obstacle, it will begin to experience a greater shearing force and will eventually be move back into the flow stream where it will undergo step 2 again, leading to the overall trapping of particles at the obstacle[77].

This result of this investigation was that by understanding hydrodynamic interactions as well as biofilms, geometries can be chose to maximise bacterial trapping times and thus improve filtering ability[77].

8 Conclusion

Investigations into biofilms have yielded indispensable information for numerous fields of study. We have explored microswimmer properties from their form in real life and how they move to how their behaviour is modelled and understood in the laboratory or simulation. To expand upon this research we looked at the oscillatory motion of microswimmers under confined geometries and their tendency to accumulate at surfaces. This lead to a detailed investigation into biofilms from how they form under different environments as well as the properties that make them invaluable to the scientific community.
References


