Developing tractable model systems for filamentous bacteria in wastewater treatment.

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The activated sludge (AS) process in wastewater treatment (WWT) is arguably ***the most important biotechnological application in the world***. Each day, large volumes of influent wastewater are processed in municipal AS plants so that safe effluent is returned to the water cycle. It is the metabolic activity of ***flocculating*** bacteria that make this process work. In short, the bacteria degrade dissolved pollutants in the wastewater whilst forming compact flocs, which are then separated from the liquid under gravity. Understanding the interactions between the bacteria however is nontrivial because any one AS plant contains 10^{18} bacteria belonging to hundreds of different species. As a result, despite significant interdisciplinary endeavour from engineers and biologists, developing tractable model systems describing the flocculating bacterial populations has proved rather difficult. Much insight has instead been gained from cultivation-independent molecular methods, or by incubating AS plant samples with wastewater in lab-scale bioreactors. The inoculum for these bioreactor experiments consists of an overwhelmingly complex microbial composition that varies between geographical locations. Consequently, these black-box experiments do little for developing a systematic understanding of AS flocs, and there is therefore a pressing need for ***model systems***.

AS flocs consist of micron-sized bacteria “glued” together by “sticky” polymers. Understanding the interactions underpinning polymer-mediated bacterial (colloidal) aggregation lies in the domain of soft matter. Therefore, the student will use the principles of soft matter to develop a simple experimental model for AS bacteria, with a particular emphasis on ***filamentous cells***. These are crucial for floc strength and structural integrity, however, too many filaments disrupt floc structure and give rise to a diffuse network that prevents compaction and subsequent settling in a process known as ***filamentous sludge bulking (FSB)***. Lab-scale bioreactor studies have contributed to the formulation of theories describing FSB but there has been little effort to develop an experimental model system to understand this phenomenon, and the aggregate/biofilm-forming behaviour of filamentous bacteria more generally. To this end, the student will use *Comamonas denitrificans* as a potential model system for WWT bacteria. *C. denitrificans*, an important denitrifier in AS, is easy to culture, has a propensity to filament, and forms aggregates in suspension and on surfaces. Our preliminary results show that this bacteria forms gel-like structures in suspension that are strongly influenced by the length of the filaments, which in turn can be tuned by the dissolved oxygen concentration. It also forms biofilms that exhibit interesting rheological properties, e.g., two-step yielding.

In this project, the student will design and perform experiments (e.g., flow cells, microfluidics, and microscopy) to characterise the structure and dynamics of filamentous *C. denitrificans* biofilms and suspended aggregates. The student will also have access to a suite of state-of-the-art techniques housed at Edinburgh such as rheoimaging, cryo FIB SEM, and liquid-state AFM, that can be used to probe the mechanical interactions involved. If the student desires, there is also scope to perform computer simulations to gain further insight into the experimental model systems.

Throughout the project, the student will have the opportunity to interact with WWT operators at Veolia UK, who manage the WWT facilities in Edinburgh, with scope to perform experiments on AS samples taken directly from these facilities. As a result, they will learn to assess and characterise how processing parameters (e.g., temperature, pH, and ionicity) influence filamentation and floc formation in the AS process, whilst using the knowledge attained through their university research to interpret the results. Through these interactions, the student will gain valuable experience working with a multinational large-scale organisation, in which they will learn to communicate scientific results in a manner that is suitable for nonacademic collaborators, and importantly, the student will also gain experience framing research questions in the context of industrially relevant problems.