

## Technical note

# Development of a gradient tube method for examining microbial population structures in floating sulphur biofilms

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## ABSTRACT

Floating biofilms occur in thin layers of between 50 µm and 500 µm on the surface of certain organic, sulphidic aquatic environments and, at times, may only be several cells deep. While these structures may be important in terms of energy flow pathways, and possibly also in wastewater treatment operations, little is known about their structural/functional properties. This is due, in part, to their flimsy nature but also to methodological constraints related to their sampling and manipulation. We have investigated floating sulphur biofilms that appear as white layers on the surface of anoxic sulphidic organic wastewaters and describe here the development of a novel gradient tube method for investigating these systems. This approach enables testing of the hypothesis that these floating sulphur biofilms are complex well-differentiated structures rather than disordered dispersions of microbial biomass as has been previously thought. Furthermore, if the former is correct, they would seem to resemble the structure and functionality of comparable complex biofilms that are attached to solid substrates. The gradient tube method involves the establishment of opposing gradients of sulphide and oxygen that are expanded across a tube of agarose 10 cm in length; this simulates the oxic/anoxic interface that occurs over only several micrometres in the natural biofilm system. A plug of sulphide-enriched agarose is first placed in the base of the tube. Samples of the floating sulphur biofilm are then mixed into agarose growth medium and, before it sets, this is overlaid on top of the plug. The tubes are then open capped and incubated. A variety of different microbial populations may thus become established in the separate physiological niches that are set up in this way within the gradient tube. The populations may be quite robustly sampled by extruding and then sectioning the agarose plug. This expansion of the biofilm enables more detailed molecular phylogenetic studies of the populations found in the various niches within the biofilm and also measurement of physico-chemical parameters within the system.

**Keywords:** gradient tube method, floating biofilms, floating sulphur biofilms, microbial ecology, sulphur biotechnology, acid mine drainage wastewaters

## INTRODUCTION

The examination of the population structure of biofilms, and particularly attempts to correlate structure and function in a way that rigorously represents the actual situation within the biofilm, remains an exacting methodological task. The requirements are particularly acute when studying floating biofilms that occur as layers of around 50 µm to 500 µm on water surfaces in some aquatic environments and are, during the early stages of formation, possibly only several cells deep (Gilfillan, 2000).

We have examined floating sulphur biofilms that form at the oxic/anoxic interface on the surface of tannery evaporation ponds (Fig. 1A), which receive high organic and sulphide loads (Gilfillan, 2000; Bowker, 2002). Similar structures have been observed in other sulphidic aquatic environments such as brackish and salt marshes and these biofilms may be an important component in total energy flow pathways in these systems (Whitcomb, 1989). While it has been suggested that they are simple accumulations of microbial growth, we have

found that these floating sulphur biofilms are composed of both obligate aerobic and anaerobic populations, including sulphide oxidising bacteria, which suggests rather that they are complex well-differentiated structures that are comparable to stratified biofilm growth found on solid substrates (Monds and O'Toole, 2009). While scanning electron microscopy of the floating sulphur biofilm (Fig. 2) clearly shows that the microbial population is embedded in an exopolysaccharide matrix that includes the production of ortho-rhombic crystals of elemental sulphur (Bowker, 2002), the possible functional differentiation of the population structure within the biofilm has remained undescribed up to the present.

The possible application of the floating sulphur biofilm system for sulphur recovery in the biological treatment of acid mine drainage wastewaters has been proposed and bioprocess development studies have been reported by Gilfillan (2000), Bowker (2002), Bowker et al. (2002), Molwantwa et al. (2007), Molwantwa (2008), Rose and Rein (2007), Molwantwa et al. (2009), Mack et al. (2009) and Van Hille et al. (2011). However, uncertainty about a biological role, if any, for sulphur formation in these films, the possible presence of differentiation within the microbial population structure and, importantly, the possibility of physiological functional differentiation within this type of system has remained unresolved. The main reasons for this are methodological constraints related to sampling and

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