



# The influence of protruding filamentous bacteria on floc stability and solid-liquid separation in the activated sludge process



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## ABSTRACT

Filamentous bacteria can impact on the physical properties of flocs in the activated sludge process assisting solid-liquid separation or inducing problems when bacteria are overabundant. While filamentous bacteria within the flocs are understood to increase floc tensile strength, the relationship between protruding external filaments, dewatering characteristics and floc stability is unclear. Here, a quantitative methodology was applied to determine the abundance of filamentous bacteria in activated sludge samples from four wastewater treatment plants. An automated image analysis procedure was applied to identify filaments and flocs and calculate the length of the protruding filamentous bacteria (PFB) relative to the floc size. The correlation between PFB and floc behavior was then assessed. Increased filament abundance was found to increase interphase drag on the settling flocs, as quantified by the hindered settling function. Additionally, increased filament abundance was correlated with a lower gel point concentration leading to poorer sludge compactability. The floc strength factor, defined as the relative change in floc size upon shearing, correlated positively with filament abundance. This influence of external protruding filamentous bacteria on floc stability is consistent with the filamentous backbone theory, where filamentous bacteria within flocs increase floc resistance to shear-induced breakup. A qualitative correlation was also observed between protruding and internal filamentous structure. This study confirms that filamentous bacteria are necessary to enhance floc stability but if excessively abundant will adversely affect solid-liquid separation. The tools developed here will allow quantitative analysis of filament abundance, which is an improvement on current qualitative methods and the improved method could be used to assist and optimize the operation of waste water treatment plants.

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## 1. Introduction

The successful operation of the activated sludge process relies on the physical characteristics of the activated sludge flocs (Andreadakis, 1993; Sezgin et al., 1978), many of which relate to the growth of filamentous bacteria. The size and morphology of floc particles determine the settleability of the flocs in the secondary clarifier. The compressibility of the floc material also affects the subsequent dewaterability. Filamentous bacteria influence these properties, together with the extracellular polymeric substances

(EPS) and floc-forming bacteria (zooglea) (Örmeci and Vesilind, 2000; Sezgin et al., 1978); these three factors therefore impact on the efficiency of the waste water treatment process (Lau et al., 1984).

The surface and structural characteristics of the activated sludge flocs are also responsible for many problems encountered during solid-liquid separation (Gray, 2004; Sheng et al., 2006). In particular, excessive growth of filamentous bacteria can result from operational changes or changes to influent composition. When such excessive growth occurs, the filaments protrude from the confines of the flocs and can link flocs in a phenomenon known as bridging. In other cases, excessive filamentous growth results in the formation of thin and open structured flocs in a process known as diffuse growth (Andreadakis, 1993; Gray, 2004). In both cases, excessive filamentous growth interferes with the solid-liquid

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separation process by producing a sludge that settles slowly and compacts poorly (Sezgin et al., 1978), commonly referred to as filamentous bulking.

In the wastewater industry, the settling and dewatering properties of a sludge containing high or low amounts of filaments are normally determined by empirical and semi-empirical parameters, such as the Sludge Volume Index (SVI). A more robust characterization of the dewatering properties can be provided by compressional dewatering theory, where the settling sludge reaches a critical solids concentration, the gel point ( $\phi_g$ ), at which solids form a network structure (Buscall and White, 1987). The settling rate of the sludge depends on the hydrodynamic interactions of the settling flocs, which can be quantified in terms of the hindered settling function,  $R(\phi)$  (Lester et al., 2005). The hindered settling function, for solids volume fractions between the initial solids concentration and gel point, can be determined from batch-settling data by means of the analytical method presented by Lester et al. (2005).

The abundance of filamentous bacteria is typically measured in the industry in a qualitative way by applying the filament ranking scheme proposed by Jenkins et al. (2004). Manual techniques, employing a microscope fitted with an ocular micrometer scale, have been applied to investigate the relationship between filament abundance and settling characteristics (Sezgin et al., 1978). More recently, image analysis has also been applied to measure the total filament length per image, which correlated well with the commonly used empirical measures for sludge settleability (Arelli et al., 2009; da Motta et al., 2001, 2002). The total filament length per image, however, might not be a sufficient measure to characterize the morphological properties of the activated sludge. It has subsequently been suggested that the filaments should be expressed as a relative measure to other floc properties, such as the floc aspect ratio (Arelli et al., 2009) to provide a better characterization of the extent of filamentous growth.

Floc stability is also important for the successful operation of activated sludge plants (Sheng et al., 2006) but few studies have considered the role of filamentous bacteria on floc stability. Although process plants are normally designed to minimise floc instability and breakup, zones of high shear are still prevalent (Jarvis et al., 2005). Ideally, the flocs should be strong enough to resist breakup to ensure high removal efficiencies (Jarvis et al., 2005; Parker et al., 1972) and to facilitate dewatering (Liao et al., 2002; Sezgin et al., 1978). The strength of floc particles depends on their internal binding forces and structure. Significant factors contributing to floc stability include: the surface charge and hydrophobicity of both the bacteria and EPS, the composition of the EPS, polymer entanglement and the presence of divalent cations involved in polymer bridging (Liao et al., 2002; Mikkelsen and Keiding, 2002; Sheng et al., 2006). Some studies suggest that the composition of the EPS, in particular the polysaccharide:protein ratio, is more important than the overall quantity (Sheng et al., 2006). Filamentous bacteria are also expected to contribute to floc stability.

The filament backbone theory is one model that seeks to qualitatively explain floc stability. The model states that a rigid backbone is formed by the filamentous bacteria, to which floc-forming bacteria are attached via polymer bridging involving their EPS (Lau et al., 1984; Örmeci and Vesilind, 2000; Parker, 1970; Parker et al., 1972; Sezgin et al., 1978). The filaments are believed to provide structural integrity by increasing the tensile strength of the flocs. Despite being widely accepted, the validity of the filamentous backbone theory was brought into question by Wilén et al. (2003), when these authors observed an inverse correlation between floc stability and the qualitative filament ranking of Eikelboom and Buijsen (1981). Case studies of activated sludge treatment plants,

however, in which the absence of filamentous bacteria resulted in pin flocs (Englande and Eckenfelder, 1972; Sezgin et al., 1978), are consistent with the filamentous backbone theory but further research is required to investigate the influence of filamentous bacteria on floc stability.

Although a number of studies have investigated the role of filamentous bacteria in operational problems such as bulking, the observations made to date have been largely qualitative and based on empirical parameters. The aim of this study was therefore to investigate the role of filamentous bacteria on the settling and compacting properties, as well as the stability of activated sludge flocs. Quantitative measures were applied to assess the abundance of protruding filaments together with a robust characterization of dewatering properties. An image analysis method was developed (using the program ImageJ) to quantify the abundance of protruding filamentous bacteria (PFB) on the basis of filament length relative to floc area. This method was then applied to a range of activated sludge samples to investigate the effect of filament abundance on the gel point and hindered settling function. The stability of the flocs in response to shear was also examined.

## 2. Materials and methods

### 2.1. Activated sludge samples

Activated sludge samples were collected from four activated sludge wastewater treatment plants, which process predominantly domestic wastewater, in Melbourne, Australia. These plants were the Altona Treatment Plant (sequencing batch reactors), Eastern Treatment Plant (continuous plug flow), Mt Martha Treatment Plant (continuous plug flow) and Boneo Treatment Plant (continuous plug flow). Upon receipt in the laboratory, the samples were placed in a refrigerator at 4 °C. Sample characterization commenced immediately with the determination of individual floc parameters, such as the filament abundance and floc size, followed by solids concentration. Dewatering and floc stability studies were performed next. One set of samples was used to conduct the dewatering studies and all experiments were completed within 2 days of sample collection, while a different sample set was used for floc stability studies and all experiments completed within 3 days of sample collection.

### 2.2. Solids concentration and density

The concentration of total suspended solids (TSS, g/L) was measured according to standard methods (Rice and Bridgewater, 2012) and used as an assessment of solids within the activated sludge samples.

The suspension,  $\rho_{sus}$  [g/L], and liquid densities,  $\rho_{liq}$  [g/L], were measured using a calibrated pycnometer (Duran group GmbH, Germany) at room temperature in triplicate. Samples were concentrated prior to measurements of  $\rho_{sus}$  [g/L] to reduce potential errors arising from low mass fractions. The solids mass fraction,  $c$  [g/g], of the thickened samples was determined by drying the samples at 60 °C in an oven (S.E.M S.A Pty. Ltd., South Australia) until a constant mass was achieved.  $\rho_{liq}$  [g/L] measurements were performed by removing the suspended solids from the activated sludge samples by centrifuging the samples for 10 min at 3000 g, followed by filtration of the supernatant through a 0.22  $\mu\text{m}$  syringe filter (Merck Millipore Ltd., Ireland).

The measured values of  $\rho_{sus}$  [g/L],  $\rho_{liq}$  [g/L] and  $c$  [g/g] were then used to calculate the solids density,  $\rho_s$  [g/L] as follows:

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