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Exploring the relationship between viscous bulking and ammonia-oxidiser abundance in activated sludge: A comparison of conventional and IFAS systems

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ABSTRACT

This study investigated the nature of viscous sludge bulking within a molasses-fed integrated fixed-film activated sludge (IFAS) and conventional activated sludge (AS) plant by routinely measuring the total carbohydrate and protein fractions of the mixed liquor (ML). The impacts of sludge settleability and plant performance on the relative abundance of ammonia-oxidising bacteria (AOB) (*Nitrosomonas oligotropha*-cluster) were also investigated using quantitative polymerase chain reaction (qPCR). Results showed that sludge volume index (SVI) correlated positively with the amount of ML total carbohydrate in both the IFAS and traditional AS plants, highlighting the influential role that ML polysaccharide concentration plays on sludge settleability in these reactors. Results also revealed a negative relationship between the AOB/total Bacteria ratio and SVI, demonstrating that a poor settling sludge generally coincided with periods of relatively low AOB abundance. The existence of these relationships suggests that readily available organic carbon (molasses) was likely to have been present in excess in these systems. Our qPCR results also showed that concentrations of both AOB and total Bacteria genomic copies detected within the ML of the IFAS and conventional AS plants were remarkably similar. For the IFAS system, results showed that the ML supported an equivalent number of AOB (per gram of biomass) to that detected on the plastic IFAS media carriers, suggesting that the suspended biomass fraction plays an equally important role in the overall nitrification performance of these systems. Interestingly, large observed variations in AOB and AOB/total Bacteria ratio measured within both the ML and IFAS media carriers had no measurable impact on the apparent nitrification performance of these systems; indicating the presence of some excess or 'reserve' nitrifying capacity above that which is required for effective plant performance. Results presented here also constitute the first known side-by-side comparison of the distribution of AOB in IFAS and conventional racetrack-like AS plants at the full-scale level.

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1. Introduction

The efficiency of the activated sludge (AS) process is governed by the effective breakdown of particulate and dissolved organics into carbon dioxide, water and new microbial biomass, and the efficient removal of this new biomass in a secondary settling tank (Horan and Shanmugan, 1986). A large proportion of the process problems experienced during AS treatment stem from an operational problem known as 'bulking sludge' (Martins et al., 2004) that ultimately results in poor solid–liquid separation in the secondary clarifiers. Sludge bulking occurs when suspended aggregates form a non-compact, low density floc (Lau et al., 1984) and is problematic because it adversely affects the separation of mixed liquor (ML) into the concentrated sludge and clarified effluent fractions—resulting in turbidity breakthrough and poor treatment performance. This poor solids separation efficiency can lead to high concentrations of suspended solids (SS) and biochemical oxygen demand (BOD_5) in the treated effluent; something which can then impact negatively on wastewater recyclability as well as the receiving environment. In addition, sludge bulking can lead to a so-called "washout" of active biomass from the AS reactor (Novak et al., 1994; Peng et al., 2003), further restricting biological nutrient removal potential and overall process performance.

Two major types of activated sludge bulking have been previously identified. The most common, and hence most widely investigated of these causes, is a process known as 'filamentous bulking' whereby filamentous organisms develop under sub-optimal conditions such as low dissolved oxygen (DO) and low food-to-microorganisms ratio (Sezgin, 1982; Wilén and Balmér, 1999; Metcalf and Eddy, 2002). The other type of sludge bulking is termed 'viscous bulking' (also referred to as non-filamentous bulking) and is characterised by the excessive growth of *Zoogloea*-like microorganisms and the production of excessive amounts of extracellular polymeric substances (EPS) (Novak et al., 1993; Peng et al., 2003). Viscous bulking has been shown to have a negative influence on the compressibility of ML due to the water-retentive nature of EPS making the specific gravity of the AS floc closer to that of the surrounding water (Novak et al., 1993; Shin et al., 2001; Mara and Horan, 2003; Peng et al., 2003; Raszka et al., 2006), with severe instances of viscous bulking shown to result in almost no solids separation (Jenkins et al., 2004).

Recent evidence suggests that the quantity and composition of EPS produced by microbial communities is dependent on a number of factors, such as microbial species composition, growth phase, the type of limiting substrate (carbon, nitrogen and phosphorous), oxygen limitation, ionic strength, culture temperature and hydrodynamic shear force (Liu et al., 2004). *Zoogloea*-like microorganisms have been suggested to be the dominant producers of EPS in AS reactors (Novak et al., 1993). Excessive growth of *Zoogloea* causes ML to have a voluminous character of jelly-like consistency, resulting in foaming and scum formation; however, excessive EPS production in AS systems is not always associated with zoogloeal growth (e.g., Jenkins et al., 2004). Nutrient limitation (nitrogen and phosphorous) is also known to have a major influence on the polysaccharide composition of activated sludge ML and, hence, can also be a contributing factor toward the occurrence of viscous bulking (Stover, 1980; Durmaz and Sanin, 2001; Jobbágy et al., 2002). For example, when the carbon-to-nitrogen (C/N) ratio is excessively high, carbon utilisation by the heterotrophic microbial population can shift from the normal state to one of luxury polysaccharide production. This was confirmed by the early work of Stover (1980) who showed that high yields of polysaccharide were most evident in AS plants when organic carbon was present in excess. Durmaz and Sanin (2001) also reported similar observations when varying the C/N ratio of bench-scale AS reactors. The same authors later reported that C/N ratio can significantly affect the physical and chemical properties of the sludge by controlling the nature and composition of the EPS fraction, with high C/N ratios leading to high concentrations of carbohydrate (EPS) production (Durmaz and Sanin, 2003).

A 2005 to 2006 survey on the settleability of ML sampled from one of South Australia's largest activated sludge plants (located at Glenelg) showed that bulking sludge, as defined by a sludge volume index (SVI) greater than 150 (Jenkins et al., 1993; Mara and Horan, 2003), was a regular occurrence. Routine microscopic examinations during this time period revealed that filamentous growth was not always prevalent and, therefore, was not always the causative factor behind the sludge bulking events. This finding highlighted the need for further investigations into the cause of these sludge bulking episodes and serves as the basis for this study. Because the feed wastewater for the Glenelg AS plant is generally carbon-limited, the process relies on the addition of an external carbon source (molasses) to drive pre-denitrification. At the time of this study, however, there was no mechanism in place for continuous regulation of the molasses dose rate (i.e. only partial flow-paced dosing during a given 24 hour period) and consequently the Glenelg AS plant has been susceptible to frequent carbon over-dosing whereby high carbon loads are unnecessarily applied during periods of low wastewater inflow. Prior to this study it was hypothesised that these instances of periodically high wastewater C/N may be responsible for the regular viscous bulking events, during which time the microbial community might have inadvertently been stimulated to generate large quantities of EPS.

The aim of this study was to investigate the nature of viscous bulking events by routinely monitoring the total polysaccharide (measured as total carbohydrate) and protein concentrations of ML sampled from Glenelg's full-scale AS plants. To further probe the physical and microbial characteristics of the Glenelg sludge, results were compared with those obtained from another local AS plant (located at Bolivar, South Australia) that did not rely on additional carbon dosing and has historically displayed much better sludge settling characteristics. Following this, the impact of sludge settleability and AS plant performance on the abundance of ammonia-oxidising bacteria (AOB) was investigated using quantitative molecular techniques. Results presented as part of this study are unique in that they provide the first known side-by-side comparison of the distribution of AOB in IFAS and conventional race track configuration AS plants receiving the same influent wastewater.