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Aerobic sludge granulation: A tale of two polysaccharides?

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ABSTRACT

Aerobic sludge granules are suspended biofilms with the potential to reduce the cost and footprint of secondary wastewater treatment. Attempts to answer how and why they form leads to a consideration of the role of their extracellular polymeric substances (EPS) in determining their physical and microbiological properties. The exopolysaccharide components of this matrix, in particular, have received attention as putative structural, gel-forming agents. Two quite different exopolysaccharides have been proposed as the gel-forming constituents, with their gel properties clearly different from those of activated sludge EPS. This review aims to address the question of whether more than one gel-forming exopolysaccharide exist in granules. Based on the available structural data, it seems likely that they are different gel-forming polymers and their differences are not artifacts of the analytical methods used. Nonetheless, both proposed structural gel polymers are extracted and purified based on procedures selecting for anionic polar polysaccharides soluble at high pH, and both contain hexuronic acids. Granulation does not result from EPS synthesis by any single microbial population, nor from production of a single exopolysaccharide. Future studies using solvents suitable for recalcitrant polysaccharides are likely to reveal important structural roles for other polysaccharides. It is hoped that this article will serve as a guide for subsequent studies into understanding the roles of exopolysaccharides in aerobic granular sludge.

1. Introduction

The application of aerobic sludge granules in wastewater treatment processes promises high rates of nutrient and organic carbon removal and a rapid separation of biomass and effluent liquid phases (de Kreuk et al., 2005; Liu and Tay, 2004). Granules seem to be an attractive, low-cost and low-footprint alternative to the now almost a century old flocculated sludge processes (Adav et al., 2008). Aerobic granular sludge has attracted considerable interest in recent years from research groups and design engineers alike (Bruin et al., 2004), and it is expected that the number of installations exploiting it will increase rapidly. Reluctance to accept this technology is largely from perceptions of low granule stability and long start-up times (Lee et al., 2010; Pijuan et al., 2011), notwithstanding that several large-scale reactors have now been operated in Europe and Africa where stable granule population has been maintained and fully compliant effluent levels achieved (www.dhv.com/nereda).

Flocculent sludge is believed to be the precursor of aerobic sludge granules, and fears of low granule stability reflect their reported propensity to revert to a floccular structure

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(Barr et al., 2010; Lee et al., 2010). It is important therefore to understand how and why these two microbial aggregates differ. Effort has been directed at comparing the physical and chemical attributes of their matrices, both of which consist of largely chemically undefined extracellular polymeric substances (EPS), to find the answer. Differences in the concentrations and distribution of proteins and polysaccharides, and levels of hydrophobicity have been cited as important (McSwain et al., 2005). A popular explanation has been that granular EPS is more adhesive than floccular EPS. Seviour et al. (2009a) provided a theoretical basis for this by demonstrating, with rheological methods, that granules could be distinguished from flocs by their gel-forming EPS, an important feature that explains the different macrostructures of granules and flocs. Hence, in granules the EPS acts as a real structural gel while with flocs the EPS is more like a paste. Seeking the chemical basis for such an important physical distinction is of considerable importance.

There seems to be a general agreement that polysaccharides are the major gel-forming constituents of the granular EPS (Lin et al., 2010a; Seviour et al., 2009a). Thus, the gel-forming characteristic of their EPS that distinguishes granules from flocs was attributed to exopolysaccharides. Subsequent characterization of gel-forming exopolysaccharides has led to an apparent and seemingly substantial discrepancy of the gel-forming exopolysaccharide components identified by two independent research groups. Lin et al. (2010a) claimed that in aerobic sludge granules it is alginate-like, while Seviour et al. (2010b) described it as a complex and highly novel heteropolysaccharide previously unreported in the literature. They named it Granulan (Seviour et al., 2011).

Several questions arise. Why have two very different polymers been proposed for the gel-forming matrix constituent? Has one or both been incorrectly identified? Are these the only EPS of importance in the matrix of aerobic sludge granules? This article attempts to answer these questions, by reviewing and analyzing published data related to the structural elucidation of these two exopolysaccharides in the context of what is known about other well-characterized bacterial exopolysaccharides. Specific reference will be made to their isolation, and their functional and structural characterization. It is hoped that this article will serve as a guide for subsequent studies into understanding the roles of exopolysaccharides in aerobic granular sludge and how this information can be exploited to improve granule formation and stability.

2. Granulan versus alginate-like exopolysaccharides

2.1. *What do the isolation techniques say about the two proposed polysaccharides?*

The granules from which the exopolysaccharide Granulan was isolated were capable of performing C, N and P removal from anaerobically treated abattoir wastewater with supplementary acetate dosing (Yilmaz et al., 2008). The alginate-like exopolysaccharides (ALE) were observed in two granule types, one from a lab-scale reactor fed with acetate, the other treating a mix of abattoir and domestic wastewater. These reactors were also achieving C, N and P removal (Lin et al., 2010a,b). Interestingly, both Granulan and ALE were shown to be soluble under relatively mild alkaline conditions, an attribute that allowed their ready isolation and structural characterization. These experiences were different from those recorded by other groups that extracting EPS from granules was difficult, thought to reflect the compact or complex nature of granules (Adav and Lee, 2008). Perhaps it would be more pertinent to explain this contradiction in terms of EPS solubility under the extraction conditions used. In multi-component biopolymer mixtures it is common for one or more components to be insoluble in any given solvent (Tolstoguzov, 1991; Turgeon and Beaulieu, 2001) and many polysaccharides are not as readily soluble as Granulan or ALE. Some high MW polysaccharides form helical structures that render them highly insoluble. This is due to hydrogen bonds forming, which hold individual polymer chains together (e.g. cellulose) (Strachan, 1932). Granules comprising such exopolysaccharides would thus be expected to produce low extractable EPS yields.

Nonetheless, differences do exist in the solubility of granules, arising probably from variations in the composition and organization of their constituent EPS. Such differences may provide clues as to the structure and possible mechanisms of cross-linking of individual exopolysaccharides. Consequently, details of Granulan and ALE isolation protocols and yields, and their limitations and biases, are discussed next.

Both Seviour et al. (2010a) and Lin et al. (2010a) used a high pH regime (NaOH and Na_2CO_3, respectively) to solubilize their granules to recover Granulan and ALE. Thus, both were soluble under high pH conditions. This is consistent with the findings by Adav and Lee (2008) that a high pH extraction (with NaOH) in combination with ultrasound treatment gave the highest EPS yield of several physical and chemical extraction methods examined. Furthermore, Seviour et al. (2009a) showed undetectable levels of contamination with intracellular substances on their extracted EPS using this NaOH method and 2-keto-3-deoxyoctonate (KDO) as a marker for Gram negative bacterial cell envelope lysis. The obtained yield of Granulan and low levels of cell lysis confirmed the suitability of this high pH method for EPS extraction from granules.

It is interesting to consider why NaOH and Na_2CO_3 addition would be so effective in solubilizing these exopolysaccharides. Granulan has a pKa of approximately 9.0 as indicated by the pH at which the sensitivity of pH change to acid addition increased dramatically (Seviour et al., 2009a). This is consistent with the view that in Granulan, polymer chain cross-linking may involve a functional group such as an amine with an alkali pKa. Given that the pKa of poly-glucosamine (i.e. chitosan) is ~6.5 (Liu et al., 2005), the sugar could not be comprised exclusively of aminated hexoses. However, since the pKa of functional groups is a function of local chemical environment, it is possible, based on the pKa of other aminated organic compounds that in Granulan, β-glucosamine amino groups could have a pKa of around 9.0. Alternatively, the sol–gel transition of granule EPS could be explained by ionization of the hydroxyl group, as is seen with konjac mannan (Williams et al., 2000).