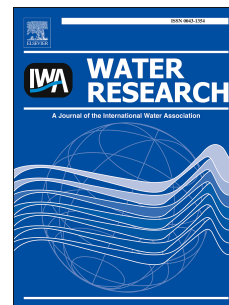


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The acid soluble extracellular polymeric substance of aerobic granular sludge dominated by *Defluviicoccus* sp.

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Keywords: *Defluviicoccus*; aerobic granular sludge; extracellular polymeric substances; biofilm; EPS extraction

Abstract

A new acid soluble extracellular polymeric substance (acid soluble EPS) was extracted from an acetate fed aerobic granular sludge reactor operated at 35 °C. Acid soluble EPS dominated granules exhibited a remarkable and distinctive tangled tubular morphology. These granules are dominated by *Defluviicoccus* Cluster II organisms. Acetic acid instead of the usually required alkaline extraction medium was needed to dissolve the granules and solubilise the polymeric matrix. The extracted acid soluble EPS was analysed and identified using various instrumental analysis including ¹H and ¹³C Nuclear Magnetic Resonance, Fourier Transform Infrared Spectroscopy and Raman spectroscopy. In addition, the glycoconjugates were characterized by fluorescence lectin-binding analysis. The acid soluble EPS is α -(1→4) linked polysaccharide, containing both glucose and galactose as monomers. There are –OCH₃ groups connected to the glucose monomer. Transmission and scanning electron microscopy (TEM, SEM) as well as confocal laser scanning microscopy (CLSM) showed that the acid soluble EPS was present as a tightly bound capsular EPS around bacterial cells ordered into a sarcinae-like growth pattern. The special granule morphology is decided by the acid soluble EPS produced by *Defluviicoccus* Cluster II organisms. This work shows that no single one method can be used to extract

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