

## Methods to Extract the Extracellular Matrix from Anaerobic Granules

J. Azeredo, A. Vilaça, C. Gonçalves, M. Henriques and R. Oliveira \*

<sup>1</sup>Centro de Engenharia Biológica - IBQF, Universidade do Minho, 4700 Braga, Portugal

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The successful operation of a UASB reactor is dependent on the formation of highly flocculated compact sludge aggregates, called granules. The structure and composition of the anaerobic granules are determinant for its organic carbon removal ability as well as for its mechanical characteristics. The granules are composed by bacteria, embedded in a matrix composed by exopolymeric substances (EPS) (polysaccharides, proteins, etc.)<sup>[2]</sup>. The EPS content is about 0.6% to 20% of the volatile suspended solids (VSS) of granules, however this value is dependent on the extraction method used. Several methods have been reported for the extraction of exopolymers from bioaggregates, including high-speed centrifugation, steaming, ultra-sonication, treatment with NaOH or EDTA, treatment with Tris/HCL buffer treatment with phosphate buffer and heat, with a cation exchange resin<sup>[3]</sup> and treatment with formaldehyde or glutaraldehyde. Many of these methods have a low efficiency in terms of selectivity of exopolymer extraction and promote cellular lysis or intracellular material loss<sup>[4]</sup>.

In this work some of the above mentioned extraction methods were assayed in order to determine their ability to extract the polymeric matrix from anaerobic granules. The extraction efficiency was determined by the yield in exopolymers and by the effect on cell lysis or intracellular material loss. The extent of cellular lysis could be evaluated by the amount of DNA extracted. Cell permeability could be inferred from a high protein content, due to the release of periplasmic proteins.

From the results it is apparent that the composition of the polymeric matrix of granules are dependent on the extraction method selected (Figure 1): Sonication extracted the greatest amount of protein and the smallest amount of polysaccharide, suggesting that some of the protein obtained was probably intracellular. The greatest amount of polysaccharide was obtained using Dowex resin and glutaraldehyde. Dowex resin is a cation exchanger resin that removes the divalent ions responsible for sludge flocculation<sup>[4]</sup>, however this method requires high speed of agitation that can promote some cellular lysis affecting especially the outer cells of granules. The smallest ratio protein/polysaccharide was obtained with glutaraldehyde. Glutaraldehyde has been used as a fixation agent to avoid the release of intracellular enzymes<sup>[5]</sup>. So, it is expected to have a minimum effect on cell lysis and cell's permeability.

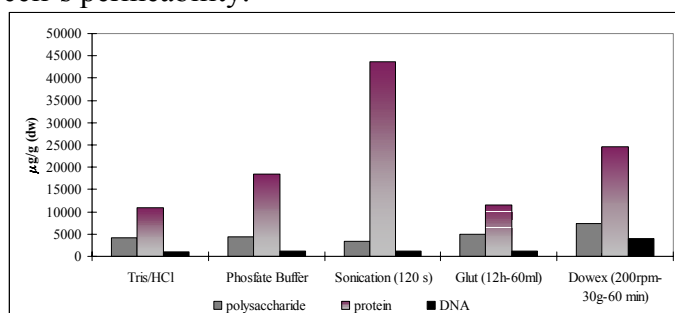


Figure 1: results of the biofilm extraction assays

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