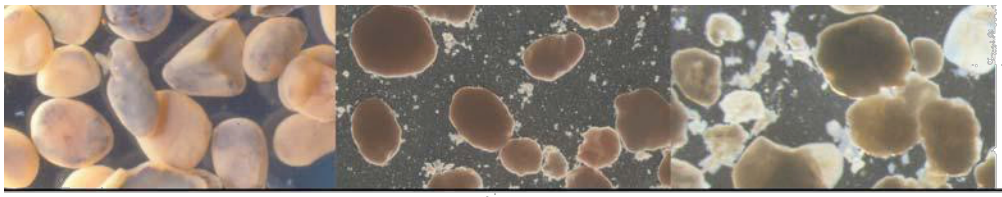

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Method evaluation of extracellular polymeric substances extraction from Anammox granular sludge with compact structure

C. Feng*, T. Lotti **, F. Malpei

* Environmental Section, Department of Civil and Environmental Engineering, Politecnico di Milano. Via Camillo Golgi 39, 20133 Milano, Italy. Email: cuijie.feng@polimi.it

**Environmental Section, Department of Civil and Environmental Engineering, Politecnico di Milano. Via Camillo Golgi 39, 20133 Milano, Italy. Email: tommaso.lotti@polimi.it

Abstract: As a major component of the matrix in granular sludge (GS), extracellular polymeric substance (EPS) is crucial in formation and stabilization of compact structure. Thus, the selection of appropriate extraction methods is of great importance, affecting the yield, composition, and the properties of EPS. However, there is still lack of the standard methods for EPS extraction. In present study, several commonly used methods were applied for extracting EPS from anaerobic ammonium oxidation (Anammox) GS, and the effectiveness of these methods were evaluated. The methods of heating, and formaldehyde with NaOH were found to obtain a higher EPS yield rate, compared to the other methods. The collected EPS was found to mainly consist of proteins (PN) and polysaccharides (PS). The high PN/PS values obtained in most methods indicated that protein content was substantially enriched in the granules. Moreover, the dominance of amide bands and/or polysaccharide -associated bands in the EPS was also revealed in the results of Attenuated total reflectance-Fourier transform infrared spectra.

Keywords: Extracellular polymeric substances; Anammox granular sludge; extraction method

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Introduction

Anaerobic ammonium oxidation (Anammox) is a promising new process to treat high-strength nitrogenous wastewater. Biogranulation is considered as an effective way to improve the low performance of the Anammox process resulted from fluctuating operational conditions and diverse inhibitors (Liu et al. 2004, Ni et al. 2010, Xing et al. 2015, Zhang et al. 2016). It has been found that Anammox bacteria can secrete extracellular polymeric substances (EPSs), and are prone to aggregate together to form stable granules even under high hydraulic shear stress (Tang et al. 2009). The aggregated GS with a larger diameter and higher density tends to settle inside reactors, favouring biomass retention thus allowing the cultivation of slow growing bacteria such as Anammox (Luet al. 2012, 2013; Chai et al. 2014; Yan et al. 2014, 2017). Hence, the EPSs are considered to be significant for formation and stabilization of granules (Liu et al. 2004).

Thus far, many procedures have been demonstrated to extract EPSs, to comprehensively study their structure, compositions, characteristics and function. The amount composition, and structure of the collected EPS quite depend on the extraction methods, including physical and chemical techniques (d'Abzac et al. 2010, Sheng et al. 2010). The main physical techniques include centrifugation, ultrasonication, heating and cation exchange resins (CER). Also, EPSs can be chemically extracted using ethylenediaminetetraacetic acid (EDTA), alkaline or aldehydic reagents However, extraction procedures are not standardized and reported results are often contradictory. Therefore, it is necessay to evaluate the EPS extraction performance of the different methods, in order to develop an optimal extraction protocol. This study aims at