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Protein and polysaccharide content of tightly and loosely bound extracellular polymeric substances and the development of a granular activated sludge floc

Mahendran Basuvaraj [a], *Jared Fein* [b], *Steven N. Liss* [a,*]

[a] *School of Environmental Studies and Department of Chemical Engineering, Queen's University, Kingston, ON, K7L 3N6, Canada*

[b] *Wastewater Department, Rothsay, Rothsay, A Division of Darling International Canada Inc., Dundas, ON, L9H5G1, Canada*

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ABSTRACT

A full-scale (FS) activated sludge system treating wastewater from a meat rendering plant with a long history of sludge management problems (pin-point flocs; >80% of floc <50 μm diameter; poor settling) was the focus of a study that entailed characterization of floc properties. This was coupled with parallel well-controlled lab-scale (LS) sequencing batch reactors (SBRs) treating the same wastewater and operated continuously over 1.5 years. Distinct differences in the proportion of proteins and polysaccharides associated with extracellular polymeric substances (EPS) were observed when comparing the properties of flocs from the FS and the LB systems. Further differences in the proportion of tightly bound (TB) and loosely bound (LB) fractions of EPS were also observed for flocs derived from conditions where differences in settling and dewatering properties of flocs occurred (i.e. FS and LS systems). FS flocs contained higher levels of EPS along with a higher proportion of LB than TB EPS, and possessing characteristics associated with non-filamentous bulking (SVI >150 mL/g). Floc formed in the LS system, following inoculation from sludge taken from the FS system, was markedly larger in size (>70% of floc >300 μm diameter), spherical in shape, compact and firm, and appeared to be granular in form. Flocs formed in the LS system, when an anoxic phase was introduced into the react stage of the SBR cycle, were found to be more hydrophobic and contained more TB and less loosely bound (LB) EPS when compared to the FS floc. TB-EPS contained a greater amount of protein, whereas the polysaccharide content of LB-EPS was larger. Protein was predominantly localized in the core region of granular flocs where cells were compactly packed. When assessing the operating conditions of the FS and LS systems parameters that appear to impact the floc

Abbreviations: CER, cation exchange resin; CLSM, confocal laser scanning microscopy; CST, capillary suction time; CSTR, continuous-flow stirred-tank reactor; DAF, dissolved air flotation; DO, dissolved oxygen; EPS, extracellular polymeric substances; ESS, effluent suspended solids; F/M, food to microorganism ratio; FS, full-scale; HRT, hydraulic retention time; LB-EPS, loosely bound-extracellular polymeric substances; LS, lab-scale; MLSS, mixed liquor suspended solids; PN, protein; PS, polysaccharide; PN/PS, protein to polysaccharide ratio; SBRs, sequencing batch reactors; sCOD, soluble chemical oxygen demand; SRT, sludge retention time; SVI, sludge volume index; TB-EPS, tightly bound-extracellular polymeric substances; WWTP, wastewater treatment plant.

* *Corresponding author*. Tel.: +1 613 533 6933; fax: +1 613 533 6934.
E-mail address: steven.liss@queensu.ca (S.N. Liss).

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properties and the transition to a granular form include dissolved oxygen (DO) concentration and food to microorganism (F/M) ratio.

1. Introduction

The link between the physicochemical and structural properties of microbial flocs and functionality in engineered systems has been a focus of numerous studies (Li and Yuan, 2002; Liao et al., 2006; Wilén et al., 2008; Van Dierdonck et al., 2013). Extracellular polymeric substances (EPS) represent an important constituent of the floc matrix contributing to the surface properties of floc, floc strength, formation of floc, as well as representing the major organic fraction in activated sludge (Mikkelsen and Keiding, 2002; Sponza, 2003; Wilén et al., 2003). EPS has been represented as an internal gel-like network but can also be characterized with respect to the association with the cell surface as either tightly bound (TB) and capsular (Seviour et al., 2012) or extending outward from the cell surface towards the surrounding environment as a loosely bound (LB) and amorphous structure (McSwain et al., 2005). Information on these two types of EPS and its constituents, and their role on bioflocculation, surface properties and sludge quality is more limited (Ahmed et al., 2007; Li and Yang, 2007; Yang and Li, 2009; Ye et al., 2011).

Previous studies indicate the role of EPS to be complex and the particular relationship between the composition, or changes in composition of EPS, and floc morphology and structure, or behaviour, and other structures including granules is unclear. There have been differences of opinion on the relationship between EPS content and bioflocculation (Chao and Keinath, 1979; Jin et al., 2003; Li et al., 2006; Liao et al., 2001). Other studies have suggested that EPS composition (Goodwin and Forster, 1985) and properties (Liao et al., 2001), rather than the quantity, are more important in sludge flocculation. More specifically, extracellular proteins have been associated with improvements in flocculation properties (Flemming and Wingender, 2001). Information about the role of EPS, and associated constituents, on granule formation, and its solid liquid separation efficiency is very limited (de Kreuk et al., 2007). The gel-like properties of granular sludge have been attributed to exopolysaccharides (Lin et al., 2010; Seviour et al., 2012), to the extent that the morphological features of granules resemble biofilms more so than flocs. For flocs a high or excessive polysaccharide content is viewed as being detrimental to settling and dewatering properties owing to a higher water content associated with polysaccharide rich EPS (Flemming, 1996); Thompson and Forster, 2003).

Proteins and polysaccharides, are major constituents which can account for 75–90% of the floc EPS mass (Tsuneda et al., 2003), with lesser amounts of humic substances, uronic acids, and nucleic acids making up the rest of the material (Liu and Fang, 2002; Sponza, 2002). EPS can be attached to the cell surface as peripheral capsules that is tightly bound (TB- EPS), or shed into the surrounding environment as a less organized (amorphous) slime, or loosely bound EPS (LB-EPS) (Comte et al., 2006). Common methods for EPS extraction usually consist of a thorough washing that strips away LB-EPS. This is typically followed by harsh extraction (Comte et al., 2006), resulting in a determination of EPS content and composition that reflects the TB-EPS content.

The present study entailed analyses of floc characteristics of a full-scale (FS) activated sludge system treating wastewater from a large meat rendering plant (Rothsay, Dundas, Ontario, Canada) experiencing varying degrees of settling and dewatering challenges. This was coupled with parallel lab-scale (LS) sequencing batch reactors (SBRs) treating the same wastewater and operated continuously over 1.5 years Flocs were generally pin flocs (<50 μm dia), and settled poorly. These structures were found to contain elevated levels of EPS, which were associated with non-filamentous bulking, and often causing the deterioration of sludge handling. Laboratory scale SBRs were operated on site in parallel to the FS system to investigate operational conditions, in well-controlled experiments, and to assist the plant in addressing its sludge management challenges. This paper reports on the content and composition of TB-EPS and LB-EPS fractions of flocs derived from different sludge samples over a range of settling and dewatering properties observed. Correlative microscopy and physicochemical analyses of flocs were carried out and revealed differences between TB- and LB-EPS reflected in their relative PN-PS ratios. A transition of floc and to granular forms in the sludge of the laboratory scale reactors was observed, and this paper reports on reactor conditions and the changes in the properties of these structures with respect to EPS.

2. Materials and methods

2.1. *Operational condition of full scale and lab scale reactors*

2.1.1. *Full scale system*

The operational conditions of a full scale (FS) activated sludge treatment system receiving wastewater from a meat rendering plant is presented in Table 1. The rendering plant processes mostly beef byproducts. The primary treatment consists of a two-step solids removal process, mechanical screening of course solids followed by finer screenings; as well as flow equalization and subsequent removal of floating solids (i.e. fats, oils, and grease) by dissolved air flotation (DAF) (Kerri et al., 2003). Following primary treatment, the remaining soluble and colloidal organic constituents and nutrients in the primary effluent are removed by secondary treatment which consists of conventional activated sludge treatment (Kerri et al., 2003). Aeration was by turbulent air flow provided by floating mechanical aerators. The primary and secondary solids separated from the wastewater are recycled back to the