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Characterization of activated sludge exocellular polymers using several cation-associated extraction methods

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ABSTRACT

Evaluation of prior research and preliminary investigations in our laboratory led to the development of an extraction strategy that can be used to target different cations in activated sludge floc and extract their associated extracellular polymeric substances (EPS). The methods we used were the cation exchange resin (CER) procedure, base extraction, and sulfide addition to extract EPS linked with divalent cations, Al, and Fe, respectively. A comparison of sludge cations before and after CER extraction revealed that most of Ca^{2+} and Mg^{2+} were removed while Fe and Al remained intact, suggesting that this method is highly selective for Ca^{2+} and Mg^{2+}-bound EPS. The correlation between sludge Fe and sulfide-extracted EPS was indicative of selectivity of this method for Fe-bound EPS. The base extraction was less specific than the other methods but it was the method releasing the largest amount of Al into the extract, indicating that the method extracted Al-bound EPS. Concomitantly, the composition of extracted EPS and the amino acid composition differed for the three methods, indicating that EPS associated with different metals were not the same. The change in EPS following anaerobic and aerobic digestion was also characterized by the three extraction methods. CER-extracted EPS were reduced after aerobic digestion while they changed little by anaerobic digestion. On the other hand, anaerobic digestion was associated with the decrease in sulfide-extracted EPS. These results suggest that different types of cation-EPS binding mechanisms exist in activated sludge and that each cation-associated EPS fraction imparts unique digestion characteristics to activated sludge.

1. Introduction

The structure of activated sludge flocs has been widely studied in order to develop a better understanding of bioflocculation in the activated sludge process. The structural composition of activated sludge flocs can be categorized as microorganisms (predominantly bacteria), organic matter in addition to microbial cells, and inorganic cations and anions (Eriksson and Alm, 1991; Jorand et al., 1995; Frølund et al., 1996; Higgins and Novak, 1997). From a microbiological standpoint, bioaggregates such as activated sludge flocs or biofilms are subdivided into cells and organic matter exterior to cells and the latter has been traditionally characterized as extracellular polymeric substances (EPS) or exocellular polymers (Wingender et al., 1999).

The importance of EPS in activated sludge flocs is due to the critical role EPS play in bioflocculation. It is known that EPS are responsible for forming microbial colonies and binding cells and other particulate materials together, leading to the flocculent characteristic of activated sludge (Wingender et al., 1999). The enzymatic digestion of polymeric organic substrates to simple, small molecules to be available for bacterial

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metabolism is another important characteristic associated with activated sludge EPS (Frølund et al., 1995). EPS in activated sludge are also thought to promote cell–cell recognition/communication and protect cells against harsh environmental conditions such as turbulence, dehydration, antibiotics and biocides (Wingender et al., 1999). All these studies show that EPS are a critical component in the formation of activated sludge flocs and in determining a variety of characteristics of bioaggregates.

Recent studies have also shown that EPS comprise the major organic fraction in activated sludge (Frølund et al., 1996; Münch and Pollard, 1997; Liu and Fang, 2002). For example, Frølund et al. (1996) found that active cells accounted for less than 10% of organic matter in activated sludges they investigated. Considering that such a large fraction of activated sludge is composed of EPS, it seems reasonable to assume that the fate of EPS in sludge digestion is a critical factor in determining the digestibility of sludge. Further, the impact of EPS on treatment processes such as sludge conditioning and dewatering should be also significant.

Because of the importance of EPS in floc formation and stability, a number of studies have attempted to extract and quantify EPS from activated sludge and a variety of extraction techniques have been employed for this purpose. However, it is not unusual to encounter a lack of agreement as to the composition of EPS, extraction efficiency, and the impact of extracted EPS on various sludge characteristics. Generally, differing results have been attributed to the different extraction methods and types of sludges.

It was recently proposed by Novak et al. (2003) that activated sludge flocs are comprised of different types of EPS with distinct metal ion binding characteristics. They proposed that the important exocellular biopolymer fractions in this floc model are: (a) lectin-like proteins that are linked to polysaccharides and bridged by Ca^{2+} and Mg^{2+}, (b) biopolymers that are bound to Fe, and (c) organic material linked with Al. It was found that anaerobic and aerobic digestion of a single activated sludge resulted in remarkably different cation and biopolymer release and different degrees of volatile solids (VS) destruction, leading to the suggestion that lectin-like proteins are mainly degraded under aerobic conditions while proteins associated with Fe are readily degraded under anaerobic conditions (Park et al., 2006b). It was shown that Al plays an important role in bioflocculation by improving effluent quality, but the impact of Al and the binding of organic matter on sludge digestion remains unclear (Park et al., 2006a, b).

If the above floc model is valid, it begins to explain the differences seen in earlier EPS studies because several of the commonly used extraction methods are selective for the EPS associated with specific cations in floc and therefore, materials extracted by one method would be expected to differ from that extracted by others. It can be also inferred that previous approaches to extracting EPS may lead to significantly varying results based on the process operation and the influent wastewater cation content.

To elucidate the nature and impact of the various EPS fractions, a more comprehensive approach to EPS extraction is proposed in this study. Specific objectives of this study were to examine the floc model suggested by Novak et al. (2003) and to study the digestibility of activated sludge EPS by anaerobic and aerobic digestion. The results of this research are expected to help determine floc composition and predict the fraction of organic material that can be degraded by different sludge digestion methods.

2. Proposed EPS extraction methods

The selection of EPS extraction methods was based on a review of prior research and from preliminary results obtained in our laboratory. These methods are described in the following sections.

2.1. Cation exchange resin (CER) extraction to target Ca^{2+} and Mg^{2+}-bound EPS

The addition of the strong Na^{+} form of a cationic resin is thought to remove multivalent cations that participate in cross-linking EPS within flocs, thereby releasing floc-bound EPS to the bulk solution (Nielsen and Jahn, 1999). However, Wilén et al. (2003a) postulated that this exchange mechanism mainly occurs between divalent cations and resin-Na^{+} because they observed that CER-extracted EPS could be correlated with Ca^{2+} and Mg^{2+} in floc, but not with either Al or Fe. This led us to choose the CER procedure to extract Ca^{2+} and Mg^{2+}-bound EPS.

2.2. Addition of sulfide to extract Fe-linked EPS

Nielsen and Keiding (1998) reported that the addition of sulfide to activated sludge removed $Fe^{2+/3+}$ from the floc matrix by formation of FeS, simultaneously resulting in a significant disintegration of the structural integrity of activated sludge floc. This study motivated us to use the sulfide to selectively remove Fe-associated EPS from activated sludge flocs.

2.3. Base extraction to release Al-associated EPS

Based on the solubility water chemistry of Al as a function of pH, Al can be solublized at either high pH or low pH conditions. Since much of biopolymer could be protonated and reflocculated at acidic conditions, base treatment at pH 10.5 was chosen to dissolve Al from solid floc and release Al-linked organic matter while allowing Fe to remain in solid form.

3. Materials and methods

3.1. Sludge samples

Activated sludge samples were collected from five full-scale wastewater treatment plants (plant A–E). Some of these facilities were sampled multiple times and each sampling was designated by numerical order. In order to study the fate of EPS in anaerobic and aerobic digestion, some of activated sludge samples were batch digested for 30 days both under