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Polyethyleneimine-mediated flocculation of *Shewanella oneidensis* MR-1: Impacts of cell surface appendage and polymer concentration

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ABSTRACT

In wastewater treatment plants, optimizing bacterial flocculation and bacterial sludge dewatering requires a detailed understanding of the concomitant biological and physico-chemical processes governing the action of flocculating agent on living cells. Here we investigate the interactions between polyethyleneimine (PEI, 60,000 g/mol) and *Shewanella oneidensis* MR-1 lacking or not the lipopolysaccharide (LPS) O-antigen surface structure. Flocculation tests were performed on bacteria with/without LPS O-antigen after being exposed to 0–100 mg/L PEI concentrations. Measurements of electrophoretic mobility and bacterial aggregates size were complemented by transmission electron micrographs and atomic force microscopy images. While low PEI concentrations (<20 mg/L) lead to flocculation of both bare and LPS O-antigen-decorated bacterial strains, the lysis of bacterial membranes occurred at larger polymer concentrations for the latter, which highlights the protective role of LPS O-antigen against harmful PEI-mediated membrane alterations. Depending on polymer concentration, two types of bacterial aggregates are identified: one that solely integrates bacterial cells, and another that includes both cells and cell residues resulting from lysis (membrane and/or LPS fragments, and inner cell content materials). The latter is expected to significantly contribute to water entrapping in sludge and thus lower dewatering process efficiency.

1. Introduction

The polyethyleneimine (PEI) macromolecule is used in numerous applications, ranging from coagulation/flocculation of bacterial wastewater sludge (Legrand et al., 1998) to permeabilization of gram-negative bacteria membranes (Alakomi et al., 2006) leading to bacterial lysis (Beyth et al., 2008). In municipal wastewater treatment plants, the activated sludge

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process consists in the use of bacterial flocs for digesting the wastewater organic matter. After the settling of those aggregates to yield the clarified water, most of the bacterial sludge is recycled via biological treatment processes, while the remaining sludge is conditioned with the addition of high molecular weight polymers such as PEI, and/or ferric chloride and lime (Deneux-Mustin et al., 2001). The procedure, that is followed by a mechanical dewatering step, results in the final collection of a bacterial cake containing at best about 70% water. Such a high water content leads to severe difficulties in terms of storage, and sludge biological post-treatment (incineration). In order to optimize the efficiency of dewatering procedure and reduce water content in bacterial sludge, detailed knowledge on the necessarily coupled biological and physico-chemical action modes of PEI on living bacterial cells is critically needed.

Bacterial communities in sludge wastewater are highly diversified and structurally organized in aggregates (Bourrain et al., 1999; Martins et al., 2004; Chaignon et al., 2002). Several studies pointed out that extra-cellular polymeric substances (EPS) produced by bacteria act as ligands between cells, which maintains a mechanical cohesion of the bacterial consortium (Jorand et al., 1995; Keiding and Nielsen, 1996). The presence of negative charges carried by the EPS (i.e. extra-cellular polysaccharides including lipopolysaccharide (LPS)) favors the binding of multivalent counter-ions that, in turn, bridge EPS chains belonging to adjacent cells, thus ensuring a given stability and strength of the formed flocs. These ions may further lead to an increase of the overall osmotic pressure of the system and thus to the trapping of interstitial water, which lowers dewatering treatment efficiency (Mikkelsen and Keiding, 1999; Curvers et al., 2009). In addition, the presence of extra-cellular polymeric substances seems to significantly impact sludge dewatering because EPS play a key role in controlling the erosion of bacterial flocs under shear flow conditions (Mikkelsen and Keiding, 1999). The amount of added flocculent is another important factor which impacts wastewater sludge dewatering. With increasing flocculent concentration, dewatering process is first improved before reaching a maximal efficiency (Marinetti et al., 2010). Waite (1999) suggested that increasing the concentration of high molecular weight cationic polymer in bacterial suspensions, results in an increase of floc size and an enhancement of dewatering efficiency. He further underlined that for some critical state of floc compactness, water cannot flow through pores of flocs anymore, but instead has to bypass the floc structure. Depending on the size of the bacterial aggregates, this can significantly lower the efficiency of dewatering process.

Although the types of associations between the flocculent and the sludge remain unclear, the aforementioned study suggests that there is some optimal floc size, reached for an optimal dose of polymeric flocculating agent, that leads to the best dewatering yield. Such dose, corresponding to an 'ideal coagulation state' in terms of dewatering, is not well-defined. The major reason for this is the critical lack of knowledge on the nature of/coupling between the bio-physico-chemical processes that determine the destabilization mechanisms of bacterial suspensions and the aggregates state after exposure to cationic polymers such as PEI. Many studies attempted to characterize flocs with the help of structure modeling and concept of fractal dimension (Martins et al., 2004; Chaignon et al., 2002; Guan et al., 1998) but few focus on the very action of polymeric flocculating agent on bacterial sludge. In addition, the alterations of bacterial membranes after contact with PEI and the corresponding impact on flocculation processes are hardly considered (Vaara, 1992; Nian et al., 2008). Nikaido (1989) showed that the LPS structure at the periphery of bacterial membranes exhibits a low permeability, thus acting as a protective barrier against potentially harmful (macro)molecules. Any disturbance in LPS organization was further shown to affect the overall bacterial permeability (Nikaido, 2005). The binding of PEI to LPS leads to a release of stabilizing counter-ions (Vaara, 1992; Nian et al., 2008), which, in turn, results in a disorganization and possible removal of LPS fragments. This is accompanied by a reordering of phospholipids that come in replacement of removed LPS (Mikkelsen and Keiding, 2002), which explains how PEI causes membrane permeabilization, and possible cellular material release (Arrington et al., 2003). All these elements unambiguously question the validity of studies where bacteria are viewed as hard (impermeable), stable inert particles when in contact to PEI. In that respect, interpretations of electrokinetic data on bacterial cells according to hard sphere models are necessarily approximate (Duval and Gaboriaud, 2010) and so are interpretations of PEI–cell electrostatic interactions according to standard DLVO theory (Duval et al., 2011). After PEI conditioning, the bacterial membranes may not remain intact, some inner bacterial materials may be released to the outer solution, thus resulting in major deviations from oversimplified theoretical DLVO predictions (Mikkelsen and Keiding, 2002). Finally, the effects of EPS on the flocculation of LPS-coated gram-negative bacteria remain poorly known.

In this study, we investigate the mechanisms governing the destabilization of bacterial suspensions after addition of PEI (molecular weight 60,000 g/mol). The analysis is carried out for *Shewanella oneidensis* MR-1 (Myers and Nealson, 1988), that have the particularity to produce various lengths of LPS O-antigen depending on its temperature of growth (Korenevski et al., 2002). The work aims at elucidating the effects of PEI on bacterial membrane, subsequent bacterial flocculation, and structure of formed flocs for strains exhibiting or lacking the LPS O-antigen surface structure. The analysis is based on systematic measurements of electrophoretic mobility and size of PEI-bacterial aggregates over a large range of PEI concentrations, and it is complemented by TEM and AFM images of flocs.

2. Materials and methods

2.1. Bacterial culture and cell conditioning

S. oneidensis MR-1 (Myers and Nealson, 1988) was cultured at 20 °C or 30 °C for 14–16 h in Lysogenic-Broth (LB Broth, Miller, Difco™), under agitation (160 rpm), in Erlenmeyer flasks with a volume filling ratio of 1–5 so as to maintain identical oxygenation conditions. Bacterial cells were harvested in stationary phase and washed twice in 1 mM KNO_3 solution (prepared in deionized water, Millipore-Milli-Q, 18.5 MΩ/cm) by centrifugation (15 min, 5000 G). At each stage of the preparation, the bacteria suspensions were kept at their