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The function of cation-binding agents in the enzymatic treatment of municipal sludge

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ABSTRACT

Treatment of sludge with enzymes has previously been shown to efficiently release organic matter. However, the added enzymes were partially adsorbed to, entrapped by or bound to the sludge structure. Simultaneous decrease of enzymes activities was observed. Reduced adsorption and more effective, lower, enzyme dose was achieved in sludge pre-treated with three cation-binding agents. The enzymatic solubilisation of sludge was improved by 150%, 240% and 290%, by 50 mM sodium tripolyphosphate (STPP), 25 mM citric acid (CA) or 50 mM ethylenediaminetetraacetate (EDTA), respectively. With cation binders, the lower relative enzyme dose 0.2 (13.7 mg/g total solids (TS)) released 3.5 times higher COD than enzyme dose 1 (68.5 mg/g TS) alone. In the presence of 25 mM CA, 75% added protease remained soluble. In the presence of 50 mM CA, EDTA or STPP, 50% of α-amylase and cellulase remained soluble. At 200 mM STPP, α-amylase was inactive, and the efficiency of enzymatic sludge hydrolysis decreased. CA was the most effective of the three cation-binding agents tested. It is biodegradable and can be produced endogenously by the microorganisms in sludge. CA has the greatest potential for the practical application to enhance biogas production. This paper reports on the possible mechanisms of enzymes adsorption to the sludge matrix and possible methods of decreasing the adsorption. We suggest that steric hindrances were responsible for the decreased enzymatic sludge solubilisation and that polyvalent metal ions were directly involved in adsorption of enzymes to sludge matrix. The addition of cation binders eliminated both phenomena and thereby improved the enzymatic solubilisation of sludge.

1. Introduction

The enzymatic hydrolysis of sludge has been investigated for the last three decades and a number of enzymes was reported to play an important role in a range of waste treatment applications (Karam and Nicell, 1997). Enzymes act on specific substances present in municipal sludge and therefore can improve the characteristics of the waste. Enzyme-treated sludge became more amenable to further treatment and could be easily and rapidly converted to value-added products (Karam and Nicell, 1997). Pre-treatment of mixed sludge with enzymes prior to the anaerobic digestion was shown to improve the degradation of the sludge and led to enhanced methane production (la Cour Jansen et al., 2004; Lagerkvist and Chen, 1993; Wawrzynczyk et al., 2003).

Hydrolytic enzymes break down polymeric substances (e.g. proteins, polysaccharides, lipids, lignin, DNA, RNA, humic substances) through multi-step processes. Initially, enzymes adsorb to the sludge-substrate and cleave small polymers

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that are loosely bound to the surface. The solubilisation of the more compact sludge matrix occurs at a lower rate. The solubilisation rate depends on the diffusion of the enzyme surface active site into particles of the sludge matrix (Venugopal et al., 1989). Previous studies showed that a combination of protease, lipase and endo-glycanases accelerated solubilisation of municipal sludge (Ayol, 2005; Ayol and Dentel, 2005; Roman et al., 2006; Watson et al., 2004; Wawrzynczyk et al., 2003). However, the enzymes became entrapped by, adsorbed to or bound to the sludge. The entrapment decreased the enzymes' action on the sludge but did not affect activity on added chromogenic soluble substrates (Wawrzynczyk et al., 2003).

Extra-cellular polymeric substances (EPS) are important integral components of the matrices of the sludge flocs. EPS are composed of a variety of organic substances: carbohydrates, proteins, humic substances, uronic acids, lipid compounds and deoxyribonucleic acids (Nielsen et al., 1996). The particulate organic matter within floc structure is maintained partially by metal ion bridges (Eriksson and Alm, 1991; Morgan-Sagastume and Allen, 2005). The removal of cations such as Ca^{2+}, Mg^{2+} or Fe^{3+} leads to disruption of flocs and release of proteins, carbohydrates and humic substances (Dey et al., 2006; Grotenhuis et al., 1991). This suggests that the treatment of sludge with cation-binding agents would cause a disintegration of the sludge. In fact, cation-binding agents have been used for the extraction of EPS (Froelund et al., 1996; Park and Novak, 2007; Wawrzynczyk et al., 2007). Consequently, the treatment with cation-binding agents should render the sludge a better substrate for enzymes. To this end, sodium tripolyphosphate (STPP) was tested. STPP is a solid, non-toxic inorganic compound (Wzorek, 2002). It is used in a large variety of household and industrial cleaning products, human foodstuffs and animal fodders. STPP provides a number of chemical functions, including pH buffering, sequestration of "water hardness", emulsification of oily materials and deflocculation of insoluble materials. Related to deflocculation is the property of petization that keeps finely divided particles (solids) in suspension and prevents them from re-coagulating (Wzorek, 2002).

Ethylenediaminetetraacetic acid (EDTA) and citric acid (CA) are well-known chelating agents. In this study, EDTA and CA were used as controls for STPP sequestering properties. CA and EDTA are universally used as antimicrobial and antiviral agents (Reidmiller et al., 2006; Soccol et al., 2004). CA is naturally produced by fungi belonging to *Aspergillus* sp. These filamentous fungi have been isolated from a sewage sludge, among others, and used for the production of CA (Jamal et al., 2005a, b).

For sludge treatment the cost effectiveness of enzymes is of prime importance. Therefore, the main focus of the investigation described in this paper was to understand possible mechanism(s) of enzyme inactivation due to attachment to the sludge matrix. The specific aims were to examine whether the sludge pre-treatment with STPP, EDTA or CA: (i) prevents the enzymes from being attached to the sludge surface, (ii) has an effect on the stability and activity of added enzymes and (iii) improves the enzymatic hydrolysis of the sludge.

2. Materials and methods

2.1. Sludge and reagents

Surplus waste activated sludge (bio-sludge) was obtained from a local municipal wastewater treatment plant in Lund (Sweden). The sludge was prepared as described earlier (Dey et al., 2006). For each experiment a fresh batch of sludge was used. The total solid (TS) content was fixed to 2% (20 g/l) and 4% (40 g/l) depending on the experimental procedure.

All reagents used were of analytical grade. Fatty alcohol ethoxylate (FAE, Synperonic 91/6), and a low molecular weight polypropylene glycol (PPG 4, polypropylene glycol P 400 E) were a gift from MB-Sveda, Malmö, Sweden. STPP was purchased from Sigma. CA and EDTA were supplied by Merck.

All used enzymes were of technical grade. Protease (Alcalase 2.4L), lipase (Lipolase 100L), and glycanases: dextranase (Dextranase PlusL), endo-xylanase (Pulpzyme HC), cellulase (Celluclast 1.5L), α-amylase (Termamyl 300L) were a gift from Novozymes A/S, Denmark. The specificities of the used enzymes were as described previously (Dey et al., 2006).

2.2. Enzyme treatment of sludge

The standard enzymatic treatment (SET) of sludge was carried out essentially according to the previously described method (Dey et al., 2006; Wawrzynczyk et al., 2003). The relative enzyme dose 1 was defined as 68.5 mg of total enzymes per 1 g TS of sludge. The enzymes dose was composed of a mixture A and a mixture B. The mixture A contained 12 mg of each enzyme: lipase, cellulase, α-amylase, endo-xylanase, dextranase and enzymes were suspended in 1.2 mg of PPG 4 and 0.12 mg of FAE per 1 g TS (Dey et al., 2006). The mixture B contained 8.5 mg of protease.

The relative enzyme dose 2 or 0.2 corresponded to relative enzyme dose 1 multiplied by factor 2 or 0.2, respectively.

2.3. Separation of sludge by centrifugation or by filtration

The sludges TS was adjusted to 2% and 4% (w/v). Each of the samples was treated with proportional enzyme dose, according to SET. After the enzymatic treatment, each sample was divided into two parts. One part was centrifuged at 7000*g*, 4 °C for 10 min and the second part was filtered through a glass filter (Glass Microfibre Filters, GF/A, pore size 1.6 μm, Whatman). Liquid and solid phases were kept on ice (over night) for further analysis. Soluble COD was measured only in the liquid phase in samples before and after treatment, immediately after the separation step. The TS content of the centrifuged samples was diluted with the supernatant in order to obtain the same TS as in correspondingly filtered samples.

2.4. Addition of cation-binding agents before the enzymatic treatment

STPP (450 mM stock solution, pH 9.0) was added to pre-concentrated sludge to a final concentration of 10, 50, 100, 150