Solubilisation of sludge by combined chemical and enzymatic treatment

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Accepted 13 June, 2007

In this study, the effects of cation-binding agents used alone and/or in combination with enzymes on solubilisation of municipal sludge and structure changes were investigated. Formic acid, citric acid, tartaric acid, EDTA, sodium tripolyphosphate (STPP), Zeolite A, sodium fluoride, sodium thiosulphate or sodium silicate were added to both biological and digested sludges. Citric acid (50 mmol/l) released the highest COD, amounting to 8 g/l from bio-sludge and 3 g/l from digested sludge. The highest specific dissolution rate was 0.5 g COD per mmol citric acid. COD released by STPP (50 mmol/l) was 3.3 g/l from bio-sludge and 2 g/l from digested sludge. STPP acted most efficiently to reduce suspended solids, 20% for digested and 40% for bio-sludge. The pre-treatment by the sequestering agents was followed by addition of three glycosidic enzymes. The used enzymes were more effective in hydrolysis of bio-sludge than in hydrolysis of the digested sludge. Additionally, after 4 h of incubation the remained enzymes activities in enzyme treated sludges were improved by up to 20%, indicating high stability of added enzymes.

Key words: Biosolids, cation-binding agents, enzymatic treatment, mass reduction, municipal waste water sludge.

INTRODUCTION

Sludge volumes are steadily increasing worldwide, as more and more countries begin to take care about their water resources. In Sweden, the strict legislation forbids land filling of municipal sludge and similar organic waste with high content of unspecified substances. Using sludge as a fertilizer in agriculture land is highly questioned and existing incineration plants cannot take care of all the sludge generated (Davidsson and Jansen, 2006). Therefore, different concepts have been developed to minimize the sludge production and/or to reduce an already generated sludge.

Anaerobic digestion is commonly used in Central and Northern Europe to reduce the produced sludge so the final bio-solid occupies less volume and will cost less to transport. In anaerobic digestion sludge is stabilized as a part of organic matter is degraded and methane is being produced.

The sewage sludge disintegration is an important step for the efficient reduction of sludge volumes and to improve the anaerobic digestion. Disintegration methods like mechanical, physical, chemical and biological have been described and tested in both laboratory and full-scale experiments (Odegaard, 2004). The enhancement of anaerobic digestion by either endogenous enzymes or added industrial enzymes has been demonstrated. Application of enzymes has been shown to improve sludge reduction as well as biogas production (Dohanyos et al., 1997; la Cour Jansen et al., 2004; Lagerkvist and Chen, 1993; Wawrzynczyk et al., 2003; Zabranska et al., 2006). On the other hand chelating agents e.g. EDTA (Brown and Lester, 1980; Eriksson and Alm, 1991) and EGTA (Bruus et al., 1992; Sanin and Vesilind, 2000) or sodium tripolyphosphate, STPP, (Dey et al., 2006) were used for extraction of extracellular polymeric substances (EPS) and were shown to release increased amount of proteins, carbohydrates and humates (Dey et al., 2006; Grothenhuis et al., 1991). Cation-binding agents disturb the sludge flocs structure by removing bridging ions (e.g. Ca²⁺, Mg²⁺, ...
sodium thiosulphate and Zeolite A in combination with Surplus biological sludge (bio-sludge) and digested sludge (mixed sludge digested under mesophilic conditions) were obtained from a local municipal wastewater treatment plant (WWTP) Öresundsverket in Helsingborg (Sweden). The sludge was pre-treated as described earlier (Wawrzynczyk et al., 2003). The total solids content (TS) of both sludges was 26 g/l ± 4 (2.6% ± 0.4, w/v).

 MATERIAL AND METHODS

 Sludge

 Surplus biological sludge (bio-sludge) and digested sludge (mixed sludge digested under mesophilic conditions) were obtained from a local municipal wastewater treatment plant (WWTP) Öresundsverket in Helsingborg (Sweden). The sludge was pre-treated as described earlier (Wawrzynczyk et al., 2003). The total solids content (TS) of both sludges was 26 g/l ± 4 (2.6% ± 0.4, w/v).

 Analytical methods

 COD was determined, in the liquid phase, using cell kits from Merck. TS, SS and VS were determined in accordance with standards methods (APHA, 1995).

 The enzymatic activity was measured in both liquid and solid part of sludge using soluble chromogenic substrates according to the assay procedures from Megazyme, Ireland (Megazyme, 2006) and as described before (Wawrzynczyk et al., 2007).

 All treatments were conducted in duplicate. Each treatment sample was analysed in triplicate and the standard deviation of all analyses was always less than 5%, unless noted in the text. All reagents used were of analytical purity.

 Enzyme treatment of sludge

 The enzymatic treatment of sludge was carried out essentially according to the previously described method (Dey et al., 2006; Wawrzynczyk et al., 2003). Enzymes were a gift from Novozyme AS, Denmark. Cellulase (Celluclast 1.5 L), endo-cellulase (Novozyme 342) and alpha-amylose (Termamyl 300 L) were used separately and in combination (CNT). The CNT mixture contained all three glycosidic enzymes and they were suspended in 1.2 mg of polypropylene glycol 400 and 0.12 mg of fatty alcohol ethoxylate per 1 g TS (Dey et al., 2006).

 Addition of cation-binding agents

 Formic acid (ForA), citric acid (CitA), tartaric acid (TarA), EDTA, sodium tripolyphosphate (STPP), Zeolite A (Zeo), sodium fluoride (Flu), sodium thiosulphate (Thio) and combination of Zeolite A and sodium silicate (Kwet) (Zeo+Kwet) were added to sludge samples. The cation-binding agents were added to pre-concentrated sludge to a final concentration of 5, 10, 25 or 50 mmol/l. The pH of sludge was adjusted to 7 using 1 M NaOH or 1 M HCl depending on experimental need.

 RESULTS AND DISCUSSION

 Effect of cation-binding agents on solubilisation of bio- and digested sludges

 Generally, the treatment of sludge with cation-binding agents led to high release of organic matter (measured as COD) and high changes in suspended matter (SS). The release of COD was positively dependent on concentration of cation-binding agent, i.e. the higher the concentration the more organic matter was released. Results of treatment of bio-sludge with nine different cation-binding agents are shown in Figure 1. COD of the agents themselves was measured and subtracted from corresponding sludge samples so that presented diagrams show the release of COD from the sludge. At cation-binding agent concentration of 5 mmol/l sodium fluoride released the highest COD (4 g/l), while at 50 mmol/l concentration citric acid was the most efficient (COD 8 g/l) (Figure 1A). However, the highest specific dissolution rate of 0.5 g COD per 1 mmol/l citric acid was observed at 10 mmol/l citric acid (released COD 5.3 g/l). The highest reduction in suspended matter was observed at 50 mmol/l STPP (40%), then EDTA (~25%), and citric acid (20%) (Figure 1B). The addition of Zeolite A and sodium silicate led to increase of SS, up to 60%, as they are mineral microporous solids it is an expected phenomena.

 The addition of tested cation-binding agents removed cations such as Ca$^{2+}$, Mg$^{2+}$, Fe$^{2+}$ and Fe$^{3+}$ from the flocs structure (Wawrzynczyk et al., 2007) and therefore the flocs network was destroyed (Figure 2) what led to enhanced release of organic matter. Based on the above results the four agents (STPP, EDTA, citric acid and formic acid) were studied further.

 Treatment of digested sludge with four selected agents led to high reduction of suspended matter (26% at 50 mmol/l STPP) and to the release of organic matter (3 g/l at 50 mmol/l citric acid and 2.1 g/l at 50 mmol/l STPP) (Figure 3).

 Effect of cation-binding agents and glycosidic enzymes on sludge solubilisation

 The highest dissolution and reduction rates were observed at low concentration of cation-binding agents both for bio- and digested sludges. Therefore the effects of 5 mmol/l cation-binding agents on enzymatic sludge solubilisation were examined. It has been shown previously that addition of cation-binding agents prior enzymatic treatment of sludge improves the process of sludge hydrolysis. In presence of STPP, citric acid or EDTA enzymes were not longer adsorbing on sludge surface and enzymatic activity was not inhibited by entrapment and as a result the enzymatic hydrolysis was significantly improved (Wawrzynczyk et al., 2007).

 In this study hydrolysis of bio- and digested sludge with
Figure 1. The effect of cation-binding agents on solubilisation of organic matter from bio-sludge showed as dissolution of COD (g/l) (A), and changes in suspended matter (%) (B).

Figure 2. The sludge flocs structure: in control sludge (A), and after treatment in presence of EDTA 5 mmol/l (B), STPP 5 mmol/l (C) or citric acid 5 mmol/l (D). Scale bars in these images represent 25 μm.

Figure 3. The effect of selected cation-binding agents on solubilisation of organic matter from digested sludge showed as dissolution of COD (g/l) (A), and changes in suspended matter (%) (B).
Figure 4. The effect of enzymes mixture and selected cation-binding agents (5 mmol/l) on solubilisation of organic matter from bio-sludge showed as dissolution of COD (g/l) (A), and changes in suspended matter (%) (B); CNT – enzymes mixture of two cellulases and alpha-amylase.

Figure 5. The effect of enzymes mixture and selected cation-binding agents (5 mmol/l) on solubilisation of organic matter from digested sludge showed as dissolution of COD (g/l) (A), and changes in suspended matter (%) (B); CNT – enzymes mixture of two cellulases and alpha-amylase.

Three glycosidic enzymes was compared in and without presence of cation-binding agents. Two cellulases, endo- and exocellulase, and alpha-amylose were used alone and in combination. COD of the enzymes themselves was measured and subtracted from corresponding sludge samples so that presented diagrams show the release of COD from the sludge. The most effective treatment was achieved with enzyme mixture (all three enzymes, CNT). Enzymes applied alone did not improve sludge solubilisation, i.e. dissolution of COD and changes in SS were comparable in enzyme treated and control samples, Figure 4 and 5. Addition of small dose of cation-binding agents (5 mmol/l) significantly improved sludge solubilisation (Figure 4 and 5).

Wawrzynczyk et al. (2007) has shown that complexing agents are preventing enzymes from being entrapped on solid phase of sludge (Wawrzynczyk et al., 2007). Citric acid was the most effective agent. Citric acid is completely biodegradable and is commonly used as an antimicrobial and antiviral agent (Soccol et al., 2004). Therefore it has a potential to be used in practice. It has been shown in our group that organic matter released after treatment with citric acid and hydro-lytical enzymes can be further utilized. The released COD was converted in to volatile fatty acids (VFA), mostly acetate (Kullenberg, 2007).

The used enzymes were more effective in hydrolysis of bio-sludge than in hydrolysis of the digested sludge (Fig-
Figure 6. The distribution of alpha-amylase after enzymatic treatment (CNT) of sludge with and without addition of cation-binding agents (cpx).

The enzymes distribution and activity

The activities of enzymes in enzyme treated sludges were measured after 4 h of treatment. The alpha-amylase got (co)-entrapped into the bio- and digested sludges (Figure 6, CNT) and both enzyme and its substrate become immobilized. Therefore the ability of enzyme to get close to the substrate within the sludge flocs was quite limited and most probably the hydrolysis took place at a low rate. In the presence of the tested cation-binding agents the alpha-amylase in enzyme treated sludge was more equally distributed between the solid and the liquid phase (Figure 6, CNT+cpx). Higher recovered activity of alpha-amylase after addition of cation-binding agents could be a result of two phenomena: (i) stability of added enzymes was improved and/or (ii) it might be due to release and activation of an endogenous amylase.

The addition of cation-binding agents to both debriding and deflocculating of sludge particles (Figure 3) and improved thereby the accessibility of added enzymes (Figure 4 and 5). According to Dimock et al. the break-up of larger aggregates results in an increase of the specific surface area available for enzymatic hydrolysis (Dimock and Morgenroth, 2006).

The tested cellulases were equally distributed between solid and liquid phase with and without cation-binding agents. These cellulases have a basic molecular architecture, consisting of a catalytic core domain linked to a small carbohydrate-binding module (CBM) type-A domain (Lee et al., 2000). The CMB type-A domains are responsible for the binding of the enzymes on insoluble polysaccharides (Boraston et al., 2004). Sludge, mainly consists of insoluble substrates, and therefore the presence of such domains is crucial for successful hydrolysis.

Additionally, after 4 h of incubation the remained enzymes activities in enzyme treated bio- and digested sludge were improved by up to 20% (data not shown), indicating high stability of added enzymes.

Conclusions

(i) First the flocs structure, stabilized by central junctions of metals, was broken up by ion-binding agents (Figure 2)
(ii) Consequently some of the earlier inaccessible organic matter was exposed and was further hydrolyzed by the addition of enzymes
(iii) The increased degradation of organic matter resulted in remaining product with a higher proportion of minerals that are easier to dewater
(iv) The adsorption of enzymes into solid was reduced
(v) Enzymatic treatment was significantly improved
(vi) Citric acid was the most effective agent and it is the most feasible to be used in sludge treatment

To conclude, we aimed at the disintegration of sludge structure to improve degradation of organic matter in the digestion step, leading to higher methane production, better dewaterability, better compactation and less volume, reduced the transport costs and easier handling of the sludge after the dewatering step.

ACKNOWLEDGEMENT

This work was granted by Kemira Oyj (Finland).

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