Fate of organic matter during moderate heat treatment of sludge: kinetics of biopolymer and hydrolytic activity release and impact on sludge reduction by anaerobic digestion

D. Lefebvre, V. Dossat-Létisse, X. Lefebvre and E. Girbal-Neuhauser

ABSTRACT

Temperature-phased anaerobic digestion with a 50–70 °C pre-treatment is widely proposed for sludge. Here, such a sludge pre-treatment (65 °C) was studied against the physical, enzymatic and biodegradation processes. The soluble and particulate fractions were analysed in terms of biochemical composition and hydrolytic enzymatic activities. Two kinetics of organic matter solubilisation were observed: a rapid transfer of the weak-linked biopolymers to the water phase, including sugars, proteins or humic acid-like substances, to the water phase, followed by a slow and long-term solubilisation of proteins and humic acid-like substances. In addition, during the heat treatment a significant pool of thermostable hydrolytic enzymes including proteases, lipases and glucosidases remains active. Consequently, a global impact on organic matter was the transfer of the biodegradable chemical oxygen demand (COD) from the particulate to the soluble fraction as evaluated by the biological methane potential test. However, the total biodegradable COD content of the treated sludge remained constant. The heat process improves the bio-accessibility of the biodegradable molecules but doesn't increase the inherent sludge biodegradability, suggesting that the chemistry of the refractory proteins and humic acids seems to be the real limit to sludge digestion.

Key words | anaerobic biodegradability, biopolymers, sludge moderate heat treatment, solubilisation, thermophilic enzymes

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INTRODUCTION

The treatment and disposal of wastewater sludge is a major contributor to the operational costs of modern wastewater treatment. In the renewable energy context, anaerobic digestion appears as a promising alternative allowing carbon conversion into methane and reduction of excess sludges (Mata-Alvarez *et al.* 2000). However, bioavailability of carbon is very different from one sludge to another and previous studies indicate that biodegradation of activated sludges is greatly influenced by their organic composition and subsequent physical properties (Mottet *et al.* 2010). Activated sludge is organised in aggregates composed of bacteria and extracellular polymeric substances (EPS) having different functions in the aggregates. Proteins, polysaccharides and humic acid-like substances are the main EPS components of bacterial aggregates (Ras *et al.* 2008a) and their hydrolysis is usually known as the rate-limiting step for the mesophilic anaerobic digestion process (Wei *et al.* 2011). The hydrolytic enzymes are located inside the biological flocs, bound to the EPS matrix or connected to the bacterial outer membrane (Frohlund *et al.* 1995; Li & Chróst 2006). The fact that enzymes are immobilised in this matrix may limit the auto-hydrolysis processes of the polymers.

Consequently, various disintegration technique strategies have been widely proposed, resulting in partial solubilisation of the particulate organic matter (Weemaes & Verstraete 1998; Liu & Tay 2001). One emerging process is temperature-phased anaerobic digestion, which applies a short (2 day) 50–70 °C pre-treatment before a mesophilic digestion. Improvement of the efficiency of a subsequent thermophilic or mesophilic digestion is clearly demonstrated by

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the increase of the methane production yield (Wang *et al.* 1997). Contradictory results could explain such an effect: only an acceleration of the hydrolysis rate (Ge *et al.* 2010) or the increase of the sludge methane potential (Skiadas *et al.* 2005; Climent *et al.* 2007; Lu *et al.* 2008).

The objective of this study was to gain more insight into the effect of a moderate heat treatment on the transformation of sludge organic material. For this, the biochemical composition of the soluble and particulate fractions was determined within a heat process of activated sludge solubilisation. Enzymatic activities of great importance for hydrolysis of solubilised macromolecules were quantified since the moderate temperature of 65 °C was expected to induce the thermophilic enzymatic potential of the sludge. Finally, the particulate and soluble fractions produced all along the heat pre-treatment were characterised in terms of biodegradability through a methane production test.

MATERIALS AND METHODS

Activated sludge

Secondary activated sludge was sampled from a municipal wastewater plant with a settling pre-treatment at the head. The initial sludge content in suspended solids and volatile suspended solids were respectively 6.6 and 5.5 g L^{-1}. Total chemical oxygen demand (COD) was measured as 7.9 g O_2 L^{-1} with a soluble fraction accounting for 0.11 g O_2 L^{-1}.

Heat treatment assays

Activated sludge was introduced into a jacketed stirred glass reactor maintained at 65 °C and connected to a condenser so that the liquid volume remained constant. The medium pH was regulated at 7 by NaOH addition. Particulate and soluble fractions were separated by centrifugation at 4,000 *g* for 15 min at 4 °C.

Polymeric substances quantification

Humic acid like substances were determined using the modified Lowry method using humic acids as the standard (Frohlund *et al.* 1995). The protein measurement was performed using bicinchoninic acid reagent with bovine serum albumin as the standard (Smith *et al.* 1985). Polysaccharide content was measured by the anthrone method using glucose as the standard (Dreywood 1946). For COD balance, biopolymer concentrations were expressed in COD, using a conversion factor equal to 1.3 g COD g^{-1} for proteins, 1.1 g COD g^{-1} for humic acid-like substances and 1.14 g COD g^{-1} for sugars. These values were the average value measured on various commercial proteins, humic acids and sugars.

Enzymatic activities

Hydrolytic activities were all assayed at 65 and at 35 °C. Protease activity was measured using azocaseine as substrate. Lipase activity was assayed using *p*-nitrophenyl palmitate (pNPP, Sigma). The α- and β-glucosidase activities were measured using respectively *p*-nitrophenyl-α-D-glucopyranoside and *p*-nitrophenyl-β-D-glucopyranoside as substrates (Ras *et al.* 2008b).

Anaerobic biodegradability

Biodegradability of both soluble and particulate fractions was measured by anaerobic long-term digestion using the biological methane potential (BMP) test at 35 °C (duration higher than 60 days). For this, the maximal methane production from sludge was measured from the pressure increase in the incubation bottles and biogas analysis by chromatography (Pommier *et al.* 2007). The maximal methane production is expressed in $NmLCH_4$/gCOD. It is known that 1 g biodegradable COD gives 350 NmL CH_4. Also the biodegradable COD is calculated by dividing the BMP value by 350 NmL CH_4 and expressed in % (g biodegradable COD/total COD). The accuracy of the method was verified by the COD balance that was closed at 4%. The BMP test was accurate enough to estimate the distribution of the sludge biodegradable COD between the soluble and particulate matters.

RESULTS

Effect of moderate thermal treatment on organic matter solubilisation

Firstly, the total COD concentration remains constant along the heat treatment test. No biomineralisation and volatilisation processes occur during these heat treatment assays. The test duration was chosen to cover the range of hydraulic retention time values proposed for thermal pre-treatment prior to a sludge digestion. The impact of the heat treatment on the transformation of sludge material was first analysed in terms of solubilisation of the main organic components.

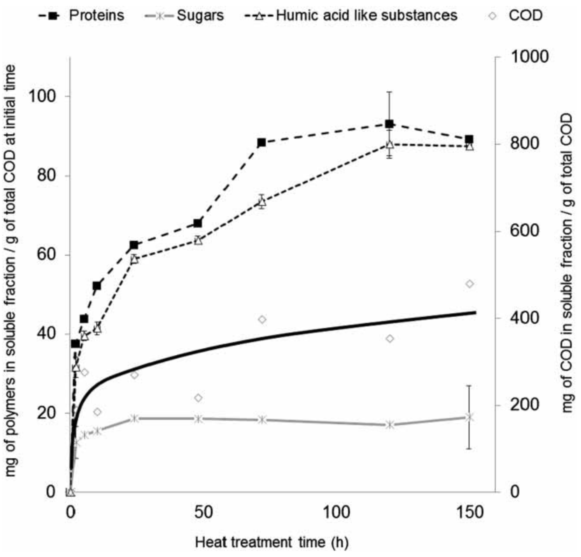

Figure 1 | Kinetics of organic matter (COD) and biopolymer (proteins, sugars and humic acid-like substances) solubilisation during activated sludge treatment at 65 °C.

Figure 1 shows a rapid solubilisation of organic matter and biopolymers during the earlier stages of the heat treatment. After 5 h at 65 °C, the sugar solubilised fractions remained nearly constant while the part of proteins and humic acid-like substances still increased, contributing to the increase of the soluble organic matter. Finally, about 50% of the sludge COD was solubilised. The major solubilisation involves protein and humic acid-like substances. At the end of the assay, the total solubilised COD is composed of 35 ± 1% proteins, 24 ± 1% humic acid-like substances and 7 ± 1% sugars. Thirty-five per cent of the soluble COD is not characterised. Certainly volatile fatty acids are not identified, in contrast to the study of Climent *et al.* (2007) performed at the same temperature. The first effect of the heat pre-treatment is clearly to promote, by solubilisation, the bioavailability of the polymers in the subsequent digester.

Characterisation of the hydrolytic activities solubilised by moderate thermal treatment

In its native state, sludge owns an intrinsic enzymatic potential. Four hydrolytic activities (protease, lipase, and α- and β-glucosidase) were measured at 65 °C and their distribution between the soluble and particulate fractions was analysed during the heat treatment. Some enzymatic activities that can be active at 65 °C were found initially in the particulate fraction as illustrated in Figure 2. A similar result was obtained for the 35 °C activities (data not shown).

Globally, the enzyme pool is active at 65 °C. Extended incubation at 65 °C leads to important reduction of these activities (around 80%), suggesting possible denaturation of these enzymes. Interestingly, some remaining activities were detected after 5 h and could be measured until 150 h. Two different enzyme behaviours were observed: the remaining thermostable lipases or proteases were slowly solubilised, and a stable and similar distribution between the soluble and particulate fractions was achieved after 24 h. For the α- and β-glucosidases associated to sugar hydrolysis, the solubilised activities drastically increased after 24 h, reaching 140% of the initial glucosidase activities after 120 h. Thus, this treatment activates the production of a significant pool of thermostable glucosidases, which suggests the presence of active thermophilic bacteria (Héry *et al.* 2010). These results point out that the heat pre-treatment reduces highly the mesophilic hydrolysis activities but can promote thermostable enzyme release into the supernatant, which may be involved in hydrolysis of sludge polymers.

Effect of the thermal pre-treatment on sludge biodegradability

The solubilisation of the sludge polymers and enzymes could promote the overall degradation process in the anaerobic mesophilic digester. For this, anaerobic biodegradability of both the soluble and particulate fractions was measured (by BMP test) at different times of the thermal treatment (Figure 3).

Even if the total biodegradable COD remained constant at about 40%, the repartition between the liquid and the solid phases drastically changed with the treatment duration. Indeed, the part of soluble biodegradable COD has increased while the fraction of the particulate biodegradable COD has decreased. Noticeably, at the end of the assay, 80% of the biodegradable COD is in the soluble form. Therefore, the positive effect of the 65 °C sludge heat pre-treatment on the enzyme and polymer solubilisation have no consequence on the maximal polymer degradation yield . The residual refractory polymers are organised in the form of thermostable aggregates. Their chemical natures are probably the real limit to their digestion (Paul *et al.* 2006). Anyway, enzyme and biopolymer solubilisation must improve digester performance by the increase of the hydrolysis kinetics as shown by Ge *et al.* (2010).

Chemical fingerprints of non-biodegradable material

Organic materials solubilised during the thermal treatment of sludge were anaerobically digested in order to study the biodegradation of each biochemical species.

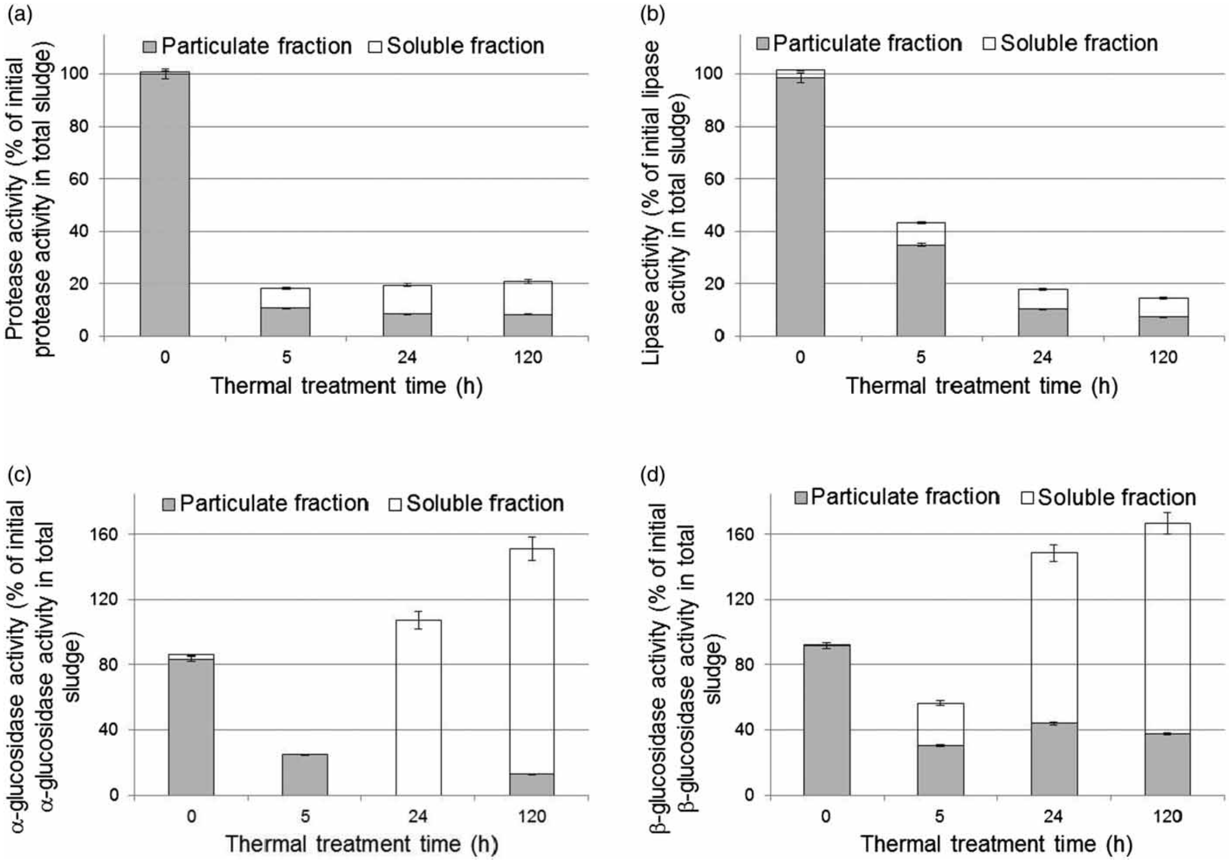

Figure 2 | Distribution of the protease (a), lipase (b), α-glucosidase (c) and β-glucosidase (d) activities in the soluble and particulate fractions during sludge treatment at 65 °C.

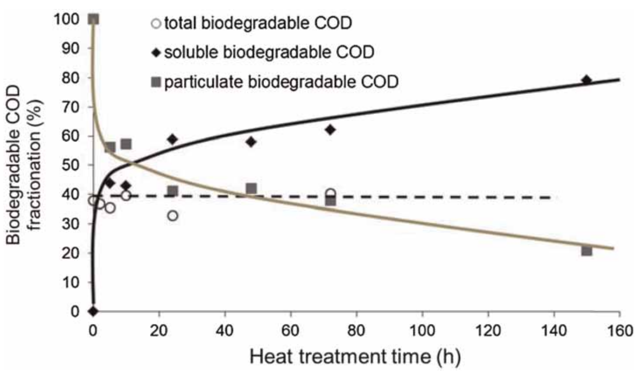

Figure 3 | Fractionation of the biodegradable COD between the soluble and particulate forms during the heat treatment.

As seen previously, the biodegradable molecules are mainly solubilised (80%). Indeed, 84% of the proteins, 91% of the humic acid-like substances and 96% of the sugars present in the supernatant were digested after a BMP test. Figure 4 illustrates the distribution of proteins, sugars and humic acid-like substances in the soluble fractions before and after anaerobic digestion (BMP test). At the beginning of the heat treatment, proteins represented 54% of polymers and this proportion slightly decreased reaching 32% after 150 h (Figure 4(a)). After anaerobic digestion, this protein fraction remained as the major non-biodegradable material (Figure 4(b)). On the other hand, sugar proportion increased from 14 to 43% during the thermal treatment but this was significantly reduced by anaerobic digestion, reaching only 12–15% of the non-biodegradable material at time 120 and 150 h. The kinetics of humic acid solubilisation was more complex and the proportion of humic acids in the soluble fraction varied from 18 to 50%, but after 24 h non-biodegradable humic acid substances accumulated in the medium in a stable proportion of 28%. After 48 h, a stable 'biochemical fingerprint' of the soluble refractory material is thus observed for the analysed biopolymers, which are 57% proteins, 28% humic acids-like substances and 15% polysaccharides.

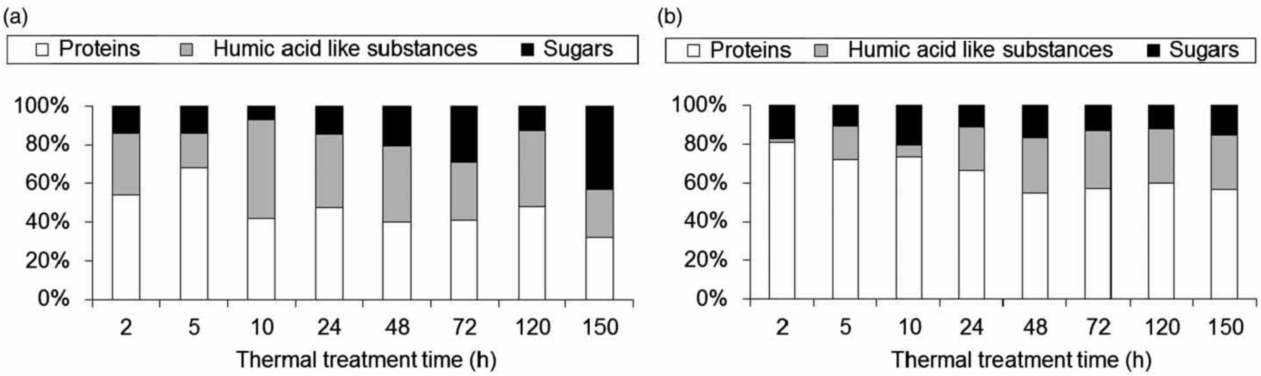

Figure 4 | Distribution of proteins, sugars and humic acid-like substances in the soluble fraction of heat-treated activated sludge before (a) and after (b) long term-anaerobic digestion.

CONCLUSION

Heat pre-treatment effect on the COD bioavailability of sludges has been widely studied against the digester performance (methane production). This study is focused on the physical, enzymatic and biological processes occurring at temperature below 100 °C. The sludge heating at moderate temperature (65 °C) evidenced two kinetics of organic matter solubilisation. The first one is responsible for rapid transfer of weak-linked biopolymers to the water phase, including either hydrophilic or hydrophobic molecules such as sugars, proteins or humic acid-like substances. However, the initial hydrolytic enzymatic pool embedded in the floc structure is partially denatured by the heat treatment. The second one induces slow and long-term solubilisation of proteins and humic acid-like substances. Such a treatment activates the selection and/or production of a significant pool of thermostable hydrolytic enzymes that may be responsible for the slight release of biopolymers during the long-term treatment. Due to the biopolymer solubilisation, the biodegradable COD is transferred from the particulate to the soluble fraction but global COD biodegradability is not enhanced and remains constant whatever the heat treatment duration. These data indicate that the chemical properties of the residual polymers are responsible for both their stable aggregation and their refractory nature. More understanding about the chemistry of the remaining refractory matter should be thus helpful to identify the enzymatic activities necessary to make these molecules biodegradable.

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