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# Cellulase Hydrolysis of Unsorted MSW

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**Abstract** A recent development in waste management and engineering has shown that the cellulase can be used for the liquefaction of organic fractions in household waste. The focus of this study was to optimize the enzyme hydrolysis of thermally treated municipal solid waste (MSW) by the addition of surfactant. Concurrently, the enzyme performance was analysed on pure cellulose in a solution of MSW wastewater. Results showed no effect of surfactant addition to the hydrolysis media as measured by viscosity and particle size distribution. MSW treatment wastewater was found to contain a high amount of calcium, potassium, sodium, chloride and others that may affect cellulolytic enzymes. Cellulase performance showed no effect of adding the metal ion-chelating agent EDTA to the solution. The cellulases were stable, tolerated and functioned in the presence of several contaminants.

**Keywords** MSW · Enzymatic hydrolysis · Cellulase · Surfactants · PEG · EDTA

## Introduction

The increasing demand for biomass as a source for fuels and chemicals has put focus on the use of waste as a biomass feedstock. An almost unexplored waste feedstock is municipal solid waste (MSW) that may provide a low-cost feedstock in large quantities. However, the use of MSW as a feedstock for bioprocesses is limited due to its heterogeneous composition of organic and inorganic components.

MSW contains very different materials such as metals, glass, textiles, minerals, inerts and many different biopolymers, which define MSW as a very heterogeneous material [1, 2]. The primary MSW treatment is sorting of the organic fraction from the inorganic fraction in order to utilise the organics for, e.g. biochemical production [3, 4]. Further fractionation of the inorganics into individual metals, glass and plastics will increase the recycling potential for these inorganic components. In this study, plastic is defined as being

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part of the inorganic waste fraction. The heterogeneous nature increases the need for sorting processes to be very robust as the MSW content is not only heterogeneous in nature; it also differs over seasons and between geographic locations [1]. Recently, we reported on a new process for the separation of organic/inorganic fractions of MSW based on enzymatic hydrolysis and liquefaction of the organic fraction, followed by simple sieving to separate organics from inorganic fractions [5].

In this process, the viscosity of the liquefied organic MSW fraction is the most important factor in order to separate the organic and inorganic fractions. MSW contains a high amount of cellulose fibres such as paper, cardboard, cellulose fabrics and vegetables [2]. Cellulose fibres have a high water-holding capacity and large molecular size. Lower viscosity upon hydrolysis of cellulose is therefore a result of smaller molecules and reduced water-bonding capacity [6]. Degradation of cellulose microfibrils into smaller fragments leads to lower viscosity and is the initial part of cellulose hydrolysis [7, 8].

Loosening and disruption of the cellulose fibril network is also needed in biofuel processes, processing, e.g. agricultural residues and fermenting released sugars [9]. The enzymatic processing of MSW is a spin-off from second-generation biofuel processes, where enzymes are one of the key process parameters for efficient sugar release and fermentation. The heterogeneous MSW material is different from the more homogenous material used in the production of second-generation biofuels, e.g. wheat straw. In addition, components in the MSW may have a negative effect on the enzymatic hydrolysis, e.g. enzyme activity, enzyme immobilization by adsorption, denaturation or competitive or non-competitive inhibition of the active site. An area of interest is therefore the variability between different biomasses and their influence on enzymatic hydrolysis. An interesting difference between the liquefaction needed for separation of MSW and the traditional process scheme of producing, e.g. bioethanol, is that complete hydrolysis to monomers is not a requirement for the separation. Hence, results from the biofuel research may not apply directly when transferred to the MSW process.

Regardless of the type of cellulosic feedstock and process, the cost and efficiency of enzymes are currently among the major factors restricting the commercialization of the biochemical conversion route for biofuel production. This is also true for MSW treatment. Many trials have demonstrated cellulase inactivation when adsorbed to non-cellulosic substances contained in the raw material like lignin or plastics [10–12]. Surfactants may reduce the unwanted adsorption of enzymes, and addition of surfactants has proven effective for enzymatic hydrolysis of various types of biomass [13]. However, the additional use of surfactants may add an extra cost to the process jeopardising the wanted cost reduction and should thereby only be used when a cost reduction is possible. Nevertheless, research in the use of surfactants for the effect on enzymatic performance on biomasses such as MSW can bring inside into the mechanisms of biomass–enzyme interactions.

The exact mechanisms of surfactants acting on biomass are still not known but the reduction of adsorption depends on the physical/surface chemistry of surfactants, enzyme and substrate. Analysis of several different surfactants in the bioethanol research has shown that only non-ionic surfactants are capable of reducing adsorption and of enhancing enzyme activity [14]. Same results have been found in experiments testing the enzymatic hydrolysis of cellulose fabrics [15]. They also found that non-ionic surfactants increased the catalytic specificity of cellulases on cellulose fabrics. Addition of 2.5–5 g polyethylene glycol (PEG) per kilogram of pretreated material during enzymatic hydrolysis of steam pretreated spruce and pretreated wheat straw revealed that the yields could be improved by 20–50% [11, 13]. An enzyme-stabilizing effect of PEG at elevated temperatures has been shown, supposedly

due to reduced inactivation through exclusion of enzymes from the lignin surface [11, 14]. The comparison of process enhancers between different enzyme–biomass processes is difficult due to biomass/substrate and process variability. However, concerning the MSW treatment the non-ionic surfactant may simultaneously have an effect on the opening of heavily burnished paper such as commercials and magazines. This non-ionic surfactant effect could increase the proportion of smaller particles after enzymatic hydrolysis compared to experiments without PEG surfactant.

Another possible obstacle reducing the enzyme performance is the abundant amounts of salt and ions in MSW [2]. Cellulases have been found to be inhibited by mercury, chloride, silver, copper, chromium, lead and zinc salts [16–18]. Exceptions exist to these general chemical inhibitions, where the level of inhibition depends on physical properties during hydrolysis and type of cellulases. The aforementioned metals may not only be inactivating but also even stimulatory for some enzyme preparations, however, mercury and chloride are strongly inhibitory [17]. Another effect of metal ions on enzymes is the polyvalent metal ions involved in direct adsorption of enzymes onto surfaces with polyvalent ions and thereby removing the enzymes from the soluble and active phase. To test the possible unfavourable effect of MSW contained ions, we used ethylenediaminetetraacetic-acid (EDTA). EDTA is a well-known ion-chelating agent used as, e.g. antimicrobial and antiviral agent.

The focus of this study was to optimize the enzyme hydrolysis of thermally treated MSW in terms of decreasing viscosity and particle size and understanding possible mechanisms of enzyme adsorption. Also included was an analysis of enzyme performance on well-characterised substrate such as filter paper in a solution of MSW wastewater. Agents used, were the non-ionic surfactant PEG and the chelating agent EDTA with the specific aims to study: [1] prevention of adsorption and inactivation of enzymes, [2] analysis of possible enzyme inhibition by contained free ions and [3] improvement of overall performance on enzymatic MSW liquefaction by these two additional agents.

## Materials and Methods

### Substrate and Substrate Pretreatment

Substrate and treatment wastewater for the trials were collected at Copenhagen municipal combustion facility, Amager Forbrænding I/S. A pilot plant is situated at Amager Forbrænding I/S treating unsorted MSW by thermal pretreatment at 95 °C for half an hour followed by enzymatic hydrolysis of organic material. The pilot plant treats 500 kg of unsorted MSW per hour in a continuous process. Substrate for the study of enzyme performance was collected after the thermal pretreatment during 2 weeks of continuous treatment. Large plastic and metal fractions were manually removed before storage in a freezer. In order to use 35% dry matter (DM) in all trials, wet fresh substrate from the pretreatment step (DM 15%) was dried at 40 °C for 48 h in an oven ensuring a dry matter content of 85%. The dried substrate was thereafter set to 35% DM by the addition of MilliQ water. The effect of free ions on enzymatic activity by utilising the ion-chelating molecule, EDTA, was studied on model cellulose, Whatman No. 1 filter paper, cut into pieces of 1 $cm^2$. The study used wastewater collected after the MSW pretreatment as reaction medium. Free wastewater from the pretreatment is recycled and reused at the pilot plant pretreatment. Recycling results in a high content of ions in the wastewater making it suitable for the study of enzyme performance.

*Substrate Compositional Analysis*

Samples were dried at 40 °C for 48 h and milled through a 1-mm screen on a Retsch ZM 200 rotor mill (Retsch GmbH, Haan, Germany). The compositional analysis was performed in duplicate as gravimetric analysis in a series of sequential steps. First step was soxhlet extraction of fat and wax on 10 g material using 500 ml chloroform for 1 h, followed by overnight drying at 75 °C. The second step was extraction of water soluble on 3.0 g of fat-free material extracted with water for half an hour at room temperature followed by dry filtration and overnight drying at 75 °C and weighed. Step three determined pectin by extraction with 3% EDTA solution, pH 4.0 for 4 h in an 85 °C water bath, followed by 500 ml hot water material wash at 50 °C and overnight drying at 75 °C and weighed. Step four determined the lignin content: Approximately 2.0 g material was stirred in 300 ml water and added 20 ml 10% acetic acid and 10 g $NaClO_2$ in 75 °C water bath for 1 h. A 10-ml 10% acetic acid and 5 g $NaClO_2$ was added after 1 h and the reaction was stopped by placing the sample in ice water. The sample was filtered through a glass filter and washed with: three times 100 ml 50 °C water, two times 100 ml 96% ethanol and one time 100 ml acetone. The filtered sample was dried at 75 °C overnight and weighed. Step four measured the hemicellulose by extraction with 50 ml 24% NaOH and 4% $H_3BO_3$ for 90 min at 25 °C followed by filtration, water wash and overnight drying at 75 °C and weighed. Sample material was weighted after each overnight drying. Cellulose was thereafter calculated as;

$$\text{gcellulose in analysis} = \text{initial weight of material} - \text{end weight of material}$$

*Wastewater Elemental Analysis*

C, H and N content were measured on dry matter that had been completely hydrolysed in acid. A 50-mg sample was opened in $H_2O_2$, $HNO_3$, HF and boric acid followed by the addition of 50.0 ml MilliQ water. C, H and N content was measured on a Thermo Finniga Organic Elemental Analyser Model Flash EA1112 (Thermo Fischer Scientific Inc., Sunnyvale, CA, USA). All the other elements described below were measured on filtrated wastewater. A 15-ml sample was centrifuged for 10 min at 4,200 RCF followed by filtration through a 0.45-μm syringe filter. Wastewater filtrate was analysed through ICP-OES for multi-element detection Cl, Al, Ca, Fe, K, Mg, Na, P, Si, As, Ba, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn. The multi-element detection was performed on a PerkinElmer, Radial ICP-OES model Optima 3,000 (PerkinElmer Inc., MA, USA) set to a sample flow rate of 1.30 ml/min, plasma gas flow of 15 l/min, nebuliser gas flow of 0.80 l/min and a plasma power of 1,300 W. Samples were diluted 5, 10 and 20 times for this analysis. Internal standards were used. $SO_4$, Cl and $NO_3$ content was measured on ion chromatograph model ICS2000 from Dionex (Dionex Inc., CA, USA) in two dilutions 10- and 50-fold.

*Enzymes*

A previous study found that cellulases were the main enzymes responsible for liquefaction of degradable MSW fractions [5]. Cellulases for this study were two commercial multicomponent cellulase preparations: Celluclast 1.5L and Cellic CTec all from Novozymes (DK). Cellulase activity was measured according to the filter paper assay [19]. The activity of these two cellulase preparations was measured to be 75 and 105 FPU for the Celluclast 1.5L and Cellic CTec, respectively. Celluclast 1.5L opposed to Cellic

CTec has almost negligible β-glucosidase activity. The β-glucosidase activity was measured to be 290 vs. 4,600 nkat/ml. β-glucosidase activity was measured as described by Wood and Bhat [20]. Celluclast 1.5L and Cellic CTec loadings were 0.5, 2 and 3.5 FPU per gram DM. The enzymes were diluted five times with sodium acetate buffer, pH 4.8, prior to addition to ensure proper mixing and injection. Enzymatic hydrolysis was stopped after 16 h by boiling each sample at 100 °C for 10 min.

## PEG and Adsorption Trials

The non-ionic surfactant, PEG, with a molecular mass of 6,000 was used to determine the surfactant effect on hydrolysis. To determine the effect of PEG on enzymatic hydrolysis of household waste, we performed small-scale treatments in 100-ml bottles. PEG was added as powder at a concentration of 1% per gram of DM (*w*/*w*). The trials were conducted inside a large heated vessel with an inner diameter of 80 cm. The vessel turned at a speed of 3 rpm ensuring a proper mixing of the 100-ml bottles and the substrate inside the bottles. The test was performed at 95°. All tests were performed in triplicate.

### *Viscosity and Particle Size Distribution*

Viscosity analysis was performed on a Brookfield DV-II Pro rotational viscometer with a full spring torque of 7,187 dyn/cm, equipped with a 20-ml sample adapter and cylindrical spindle number SC4-27 (Brookfield Engineering Lab, Middleboro, MA, USA). The sample chamber was embedded into a flow jacket kept at 25 °C. Viscosity measurements were performed on 8 g of hydrolysed slurry. Measurements were taken between 10% and 90% full spring torque. Three spindle shear rates were used (37.20/s, 39.06/s, 40.92/s) and the average of three data points were collected at each shear rate.

All samples were sieved through a 2- and a 1-mm sieve for particle size distribution measurements performed on a Fritsch vibratory sieve shaker "ANALYSETTE 3 PRO" (Fritsch GmbH, Idar-Oberstein, Germany) set to 10 min shaking at 2.0-mm amplitude. The sieves were washed and dried between each sample.

### *HPLC Analysis*

Following viscosity and particle size measurements, samples were centrifuged at 4,200 RCF for 10 min and the supernatants were used for sugar analysis. The supernatants were filtered through a 2-μm glass filter and diluted fourfold prior to sugar analysis. Sugar release was measured and quantified with a Dionex Summit HPLC system equipped with a Shimadzu RI-detector. The separation was performed with a Phenomenex Rezex ROA column at 80 °C, with 5 mM $H_2SO_4$ as the eluent, at a flow rate of 0.6 ml/min.

## Ionic Inhibition of Enzymes

The study of enzyme activity with and without metal ion chelation was performed on model cellulose in 250-ml Erlenmeyer flasks on a rotating table set to 150 rpm and 50 °C. Control samples with only EDTA and control samples with only MilliQ water or MSW waste/process water were included in the study. All treatments were performed and measured in triplicate. MSW wastewater was sieved water taken after the initial thermal treatment step at the MSW treating pilot plant. EDTA was added to the samples as a stock solution made from EDTA powder and MilliQ water. EDTA concentrations used were 50 and 200 mM.

Trial DM was 2% reached after the addition of calculated amount of MilliQ water. Amount of added MilliQ water was based on material DM and after the subtraction of possible added enzyme and/or EDTA solution. Trial pH was 4.8 for all treatments. Enzymes used were similar to enzymes used in the study on factual household waste. Enzyme loading was set to 2 FPU/g DM. Samples were boiled for 10 min after 16 h of hydrolysis, centrifuged and sieved as described above. Differences in activity were determined as sugar release between samples with a Dionex Summit HPLC sugar analysis as described above.

## Results and Discussion

### Effect of Surfactant

The MSW fibre fraction contains 11.5% lignin (Table 1) which is relatively high compared to, e.g. wheat straw [13]. Lignin has a binding affinity for cellulases and may thus reduce the efficiency of the enzymes. MSW is simultaneously a very heterogeneous material which likely contains materials with similar physical chemical surface properties as lignin. The compositional analysis also shows that the pretreated fibre fraction of MSW contains about one-third cellulose (Table 1). Consequently, cellulases are the primary enzyme responsible for liquefaction of MSW fibre fraction.

Figure 1 shows the viscosity as result of enzyme loading and addition of PEG. PEG has no effect on the viscosity of hydrolysed waste using Cellic CTec. Viscosity decrease linearly with increasing enzyme activity and there is no significant effect of added non-ionic surfactant. The picture is analogous when applying Celluclast 1.5L (Fig. 1). The linear courses of both enzymes are very similar. Cellic CTec has a higher activity per volume of enzyme solution and thereby reduces the necessary addition of enzyme. However, Cellic CTec appears to have no appreciable improvement on liquefaction as measured as a function of enzyme activity. The addition of PEG revealed no improvement in liquefaction of the degradable fraction of MSW even though 13% lignin is present (Table 1). MSW lignin may not affect the enzymes and thereby the liquefaction. Alternatively, PEG has no binding affinity for the MSW-lignin. Hence, enzymes may be bound on MSW lignin and inactivated in samples even with or without PEG. The higher β-glucosidase content of Cellic CTec compared to Celluclast 1.5L showed no improvement on liquefaction measurements. The hypothesis was that even though we are not seeking for complete hydrolysis, some product inhibition could occur in samples treated with Celluclast 1.5L effecting lower hydrolysis efficiency. There is no obvious improvement by using Cellic CTec with the higher β-glucosidase content when focusing solely on viscosity.

**Table 1** Compositional analysis of organic fibre fraction after thermal pretreatment

| Component/type | Content (% of DM) | SD |
|---|---|---|
| Fat, chloroform extractives | 12.8 | 0.0 |
| Water extractives | 20.1 | 0.5 |
| Pectin | 10.6 | 0.3 |
| Lignin | 11.5 | 0.8 |
| Hemicelluloses | 14.0 | 1.1 |
| Cellulose | 31.0 | 0.0 |

Average content and standard deviation (SD) are shown

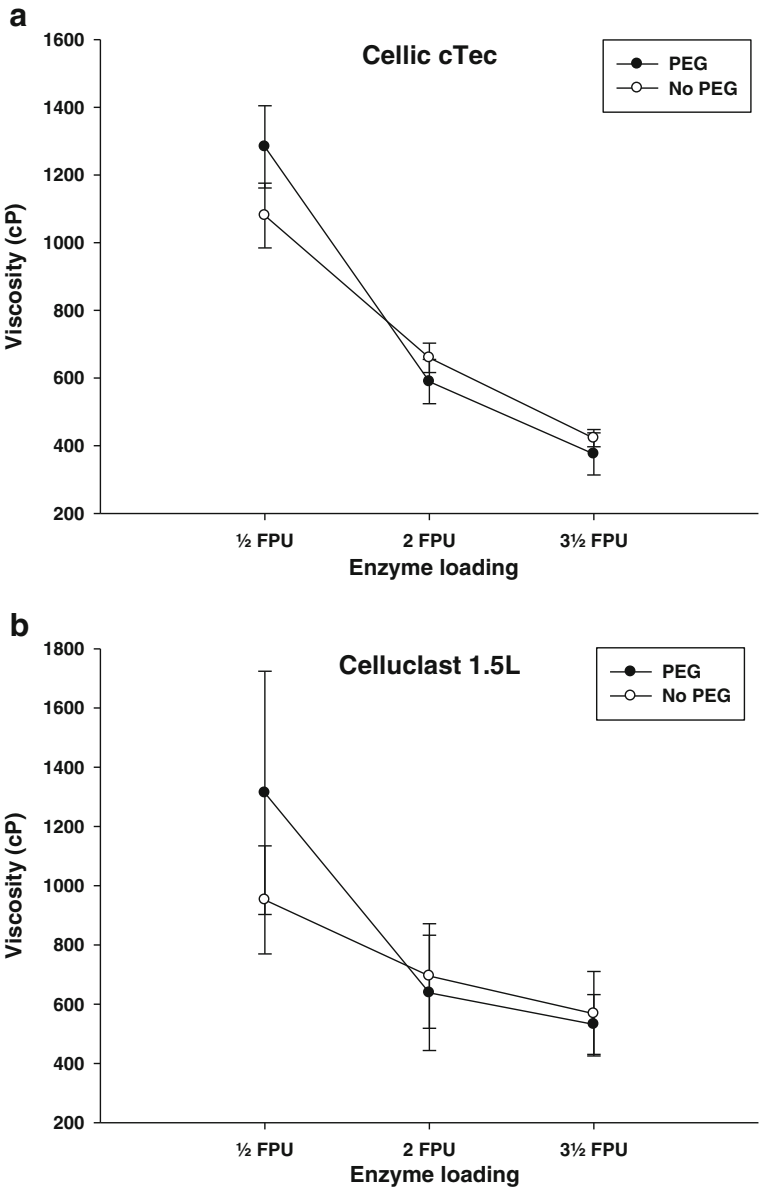


**Fig. 1** Viscosity change (*cP*) at increasing Cellic CTec (**a**) and Celluclast 1.5L (**b**) loadings (FPU/g DM) with or without the non-ionic surfactant PEG. *Filled symbols* represent the PEG added samples and the *open symbols* represent the non-PEG samples. *Error bars* represent independent measurements from different experiments ($n$=3)

The effect of PEG addition on the particle size distribution was also tested for Cellic CTec and Celluclast 1.5L (Fig. 2). Using Cellic CTec as cellulase enzyme preparation, we found no effect of PEG on particle size distribution. It seems as there is a slight difference in the proportion between 2 and 1 mm and the fraction below 1 mm with and without PEG. Again the experiments also show an analogy with the enzyme Celluclast 1.5L compared to

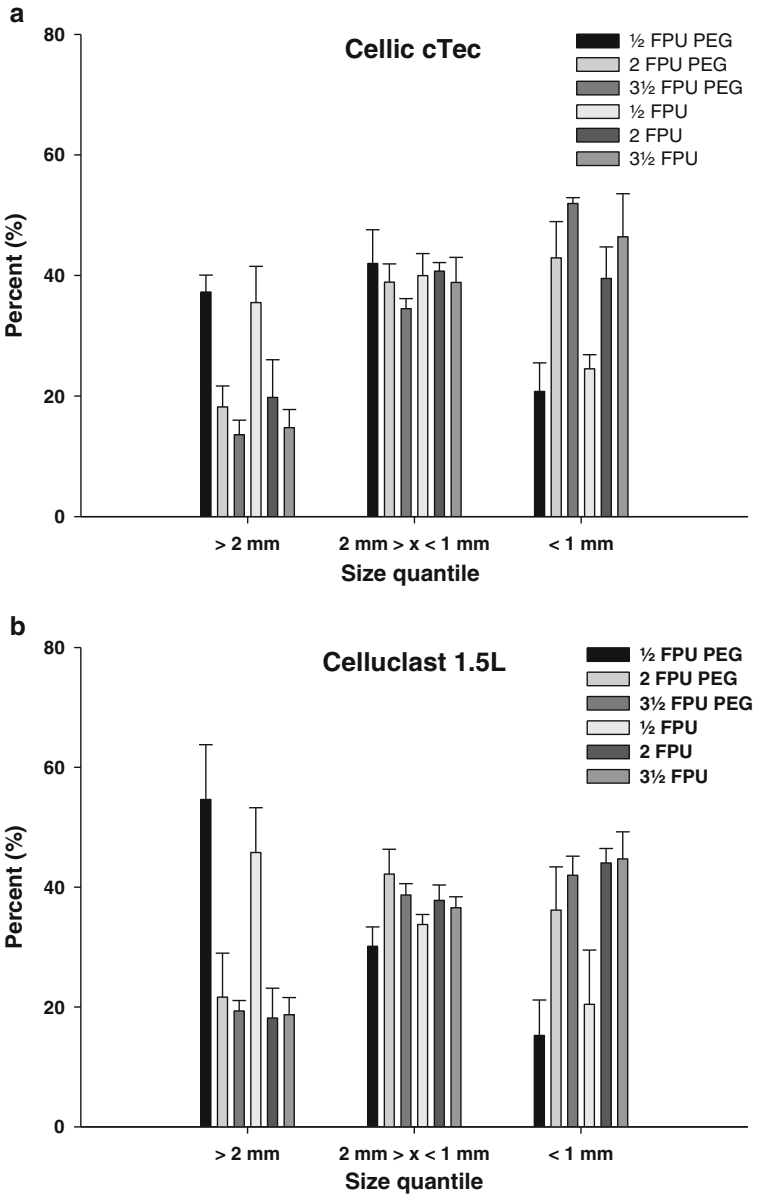


**Fig. 2** Particle size distribution for samples treated with Cellic CTec (**a**) or Celluclast 1.5L (**b**) and with or without 1% PEG 6,000. Samples are collected in sets of size quantiles. *First three vertical bars* in each set represent samples treated with PEG. *Error bars* represent independent measurements from different experiments ($n$=3)

Cellic CTec (Fig. 2). Applying Celluclast reveals a trend towards less reduction of particle size after using the non-ionic surfactant PEG. Samples without PEG have a larger fraction of particles smaller than 1 mm (about 5%) and a smaller fraction above 2 mm independent of enzyme loadings. These findings are opposite when using Cellic CTec. However, the variations are too large to find them in additional experiments. The difference between

samples with PEG and samples without PEG is more pronounced at low enzyme loadings. The difference at low enzyme loading could be a consequence of the binding and inactivation of a low amount of enzyme, which equals a larger fraction of total added enzyme. If PEG can circumvent this inactivation, it will probably show up in the samples where enzyme loading is the true bottleneck; at low enzyme loadings. However, when Celluclast 1.5L is used, we find the best performance without PEG. PEG has not shown any effect on cellulose catalytic specificity and on the opening of heavily burnished paper resulting in increased proportion of smaller particles.

The largest change in particle size distribution is a function of enzyme loading and found between 0.5 and 2 FPU/g DM enzyme loading. Comparing the two cellulase preparations in Fig. 2 reveal that Cellic CTec reduce the overall particle size and lower the proportion of particles greater than 2 mm more than Celluclast 1.5L. Cellic CTec shows a better effect on particle size distribution, again the difference is most apparent at low enzyme loadings. Celluclast 1.5L was chosen as a reference enzyme because of its capabilities for hydrolysing the degradable fraction of MSW [5]. A main difference between Celluclast 1.5L and Cellic CTec is the lack of β-glucosidase activity of Celluclast 1.5L. It is not known whether or not Cellic CTec has a different composition of exo- and endo-glucanases compared to Celluclast. A hypothesis for the difference on particle size distribution at low enzyme loadings could be cellobiose product inhibition in Celluclast. Furthermore, a different profile of exo- and endo-glucanases between the two enzyme preparations could result in differences in particle size distribution at low enzyme dosages.

HPLC analysis following the viscosity measurements are shown in Table 2. HPLC analysis is performed on the filtrate of the fraction less than 1 mm following particle size distribution measurements.

The most striking differences are the levels of cellobiose and glucose between samples treated with Cellic CTec and samples treated with Celluclast. Cellic CTec contains more β-glucosidase than Celluclast to meet the requirements for ethanol fermentation in the biofuel industry. For fermentation into bioethanol, it is imperative that cellulose is completely decomposed to monomeres. Typically, Celluclast 1.5L has always been added extra β-

**Table 2** HPLC analysis of Cellic CTec or Celluclast 1.5L hydrolysed waste samples with and without the addition of PEG

| Treatments | Cellobiose (g/l) | Glucose (g/l) | Xylose (g/l) | Lactate (g/l) | Acetate (g/l) | Ethanol (g/l) |
|---|---|---|---|---|---|---|
| ½ FPU cellic PEG | n.a. | 7.90 (0.19) | 0.17 (0.09) | 8.91 (0.57) | 6.21 (0.25) | 1.91 (0.14) |
| ½ FPU cellic | n.a. | 7.74 (0.28) | 0.75 (0.03) | 8.64 (0.69) | 6.36 (0.20) | 2.08 (0.26) |
| 2 FPU cellic PEG | n.a. | 16.25 (0.64) | 1.46 (0.09) | 8.52 (0.70) | 5.31 (0.16) | 2.74 (0.56) |
| 2 FPU cellic | n.a. | 15.52 (0.40) | 1.50 (0.06) | 8.26 (0.64) | 5.60 (0.18) | 2.19 (0.04) |
| 3½ FPU cellic PEG | n.a. | 20.94 (0.19) | 1.93 (0.06) | 7.66 (0.84) | 5.13 (0.04) | 2.84 (0.14) |
| 3½ FPU cellic | n.a. | 21.71 (0.34) | 2.04 (0.02) | 7.98 (0.74) | 5.20 (0.31) | 2.37 (0.21) |
| ½ FPU celluclast PEG | n.a. | 2.44 (0.13) | 1.11 (0.08) | 10.36 (0.57) | 6.39 (0.20) | 2.59 (0.31) |
| ½ FPU celluclast | n.a. | 2.52 (0.15) | 1.136 (0.16) | 11.37 (0.25) | 7.21 (0.17) | 2.08 (0.46) |
| 2 FPU celluclast PEG | 10.52 (0.51) | 7.59 (0.37) | 2.35 (0.15) | 11.25 (0.89) | 6.60 (0.33) | 2.77 (0.32) |
| 2 FPU celluclast | 10.12 (0.38) | 7.21 (0.24) | 2.23 (0.07) | 10.96 (0.48) | 6.27 (0.29) | 2.40 (0.13) |
| 3½ FPU celluclast PEG | 10.83 (0.56) | 10.61 (0.02) | 2.70 (0.06) | 10.46 (0.80) | 6.25 (0.11) | 2.96 (0.39) |
| 3½ FPU celluclast | 11.24 (0.57) | 10.90 (0.42) | 2.81 (0.12) | 10.79 (0.14) | 6.35 (0.26) | 2.35 (0.28) |

Numbers given in the parentheses are standard deviations, $n=3$

glucosidase in the form of Novozym188 to cover for the shortage in Celluclast 1.5L [21]. The experiments in these experimental trials show that it is not necessary to add additional β-glucosidase to Celluclast 1.5L to obtain viscosity reduction and liquefaction of the organic fraction of household waste (Fig. 1 and Table 2). HPLC results show that Cellic CTec produce more glucose as the enzyme loading increases which is an effect of the increased β-glucosidase activity. Celluclast 1.5L in contrast keeps having free cellobiose in the solution; however, the effect of free cellobiose that may inhibit exo- and endo-glucanase is not visible in the measure of viscosity.

The analysis shows more lactic acid in samples treated with Celluclast 1.5L compared with samples treated with Cellic CTec. Samples treated with Celluclast 1.5L shows higher concentration of free xylose than measured in the samples treated with Cellic CTec that may be the reason for the higher production of lactic acid. The ratio of glucose/xylose can change the metabolic pathway and thereby the product yield of lactic acid-producing bacteria [22]. The higher xylose content in Celluclast-treated samples that must be attributed to a higher xylanase activity had no effect on the viscosity or particle size distribution.

Ethanol content is increasing in trials added PEG although no visible increase in sugar levels. PEG has so immediately a positive effect on the fermentation of sugars to ethanol, which seems difficult to explain. It must be said that this fermentation is spontaneous since ethanol-producing organisms has not been added. Antimicrobial chemicals were not used in the experiment as we wanted to simulate the pilot plant processing of MSW and to look for any effect of PEG addition in this setup.

## Effect of Salts and Metal Ions on Enzymatic Activity

Prior to executing this analysis, we did an analysis of free ions in the wastewater from the pretreatment of MSW. This analysis showed that the pilot plant wastewater contains a large group of free ions shown to affect cellulases (Table 3) [16–18]. Former literature has shown mercury, copper, chromium, lead, zinc and iron to be inhibitory to cellulases. However, some of the metal ions (calcium, cobalt, magnesium and manganese) are also known to stimulate some enzyme activities [17]. MSW was found to contain high amounts of stimulatory elements such as calcium, magnesium and some manganese (Table 3). However, present is also a high content of the inhibitory element zinc and some iron. Mercury and cobalt are not present (Table 3).

The analysis of enzyme activity was performed through a decomposition of filter paper with Cellic CTec or Celluclast 1.5L in either sodium citrate buffer or wastewater from the MSW pilot plant. Both reaction media were adjusted to pH 4.8. The experiments were performed with or without EDTA solution and the EDTA solution was adjusted to pH 4.8 prior to addition. Results are shown in Table 4. EDTA shows no ameliorative effect on the enzyme hydrolysis although EDTA will bind solubilised salts and metal ions. Certain metal ions have a negative effect on enzyme activities and this negative effect is eliminated in some other studies after the addition of EDTA [23]. They tested the enzymatic treatment of municipal sludge on the release of COD from dry matter and found a significant effect after the addition of EDTA. In this study, we have used up to four times higher concentrations of EDTA, 200 mM, compared to the study performed by Wawrzyńczyk et al. [23]. Process wastewater from the pilot plant has no immediate negative effect on chosen enzymes. We can conclude that tested wastewater fraction did not contain any, for the tested enzyme solution, affecting substances. In fact, the element with highest concentration was calcium that is stimulatory to cellulases [17]. Hence, the chosen enzyme solutions are very activity

**Table 3** Analysis of content of DM, ash, and elements in MSW wastewater from pilot plant

| Parameter/element | Unit | Meas. technique | Average | Percentage of total | SD |
|---|---|---|---|---|---|
| Based on total | | | | | |
| DM | % | DS 204 | 1.8 | | 0.0 |
| Ash | % | DS 204 | 1.4 | | 0.0 |
| C | % of DM | Flash combustion, GC | 35 | | 0.0 |
| H | % of DM | Flash combustion, GC | 6.0 | | 0.0 |
| N | % of DM | Flash combustion, GC | 4.0 | | 0.2 |
| Based on filtrate | | | | | |
| Al | mg/l | Radial ICP-OES technique | 6.8 | 0.25 | 0.1 |
| Ca | mg/l | Radial ICP-OES technique | 245.3 | 8.85 | 9.2 |
| Fe | mg/l | Radial ICP-OES technique | 20.5 | 0.74 | 0.9 |
| K | mg/l | Radial ICP-OES technique | 494.0 | 17.82 | 16.4 |
| Mg | mg/l | Radial ICP-OES technique | 41.5 | 1.50 | 0.8 |
| Na | mg/l | Radial ICP-OES technique | 883.3 | 31.86 | 5.8 |
| P | mg/l | Radial ICP-OES technique | 37.1 | 1.34 | 1.2 |
| Si | mg/l | Radial ICP-OES technique | 33.2 | 1.20 | 0.3 |
| Ba | µg/l | Radial ICP-OES technique | 130.0 | 0.00 | 12.2 |
| Cr | µg/l | Radial ICP-OES technique | 120.0 | 0.00 | 2.2 |
| Cu | µg/l | Radial ICP-OES technique | 140.0 | 0.01 | 2.0 |
| Hg | µg/l | Radial ICP-OES technique | 0.0 | 0.00 | 0.0 |
| Co | µg/l | Radial ICP-OES technique | 0.0 | 0.00 | 0.0 |
| As | µg/l | Radial ICP-OES technique | <50 | 0.00 | 0.0 |
| Mn | µg/l | Radial ICP-OES technique | 480.0 | 0.02 | 2.7 |
| Ni | µg/l | Radial ICP-OES technique | 250.0 | 0.01 | 0.8 |
| Pb | µg/l | Radial ICP-OES technique | 70.0 | 0.00 | 3.3 |
| Zn | µg/l | Radial ICP-OES technique | 7,150.0 | 0.26 | 16.3 |
| $SO_4$ | mg/l | IC technique | 153.3 | 5.53 | 5.8 |
| Cl | mg/l | IC technique | 846.7 | 30.54 | 30.8 |
| $NO_3$ | mg/l | IC technique | 2.0 | 0.07 | 0.0 |

DM, ash and C, H, N and S are based on total fresh wastewater, whereas the rest are measured as solubilised elements in wastewater filtrate. Given values are average and standard deviation (SD) of three replicates

stable in terms of hydrolysing unsorted heterogeneous MSW. Runoff wastewater from the thermal pretreatment step is recycled continuously. Thus, the wastewater should be representative for comparing enzymatic activity of cellulases when hydrolysing model cellulose in either optimal buffer solution or wastewater solutions.

The test results show a higher glucose and lower cellobiose content in the samples treated with Cellic CTec (Table 4). Cellobiose is hydrolysed into glucose in the Cellic CTec samples; this effect, which is desired in the glucose fermenting processes [7, 24], has not resulted in any effect in the PEG trial as shown in the results from viscosity measurements of PEG trials (Fig. 1). Product inhibition did not reflect higher viscosity in the samples treated with Celluclast. Cellulases for liquefaction of household waste do not need additional β-glucosidase to function satisfactorily in liquefaction of household waste. Everything depends of course on post-treatment of the slurry, which may depend on the sugar content for, e.g. fermentation. The lactate has been reduced by the addition of EDTA as lactate concentrations are lower for

**Table 4** HPLC analysis after hydrolysis of filter paper for 16 h using Cellic CTec or Celluclast 1.5L in Na-citrate buffer (pH 4.8) or pilot plant wastewater (pH 4.8) and with or wtihout EDTA

| Treatments | Cellubiose (g/l) | Glucose (g/l) | Lactate (g/l) | Acetate (g/l) |
|---|---|---|---|---|
| Buffer | n.a. | n.a. | n.a. | n.a. |
| Ctec, buffer | n.a. | 3.31 (0.16) | n.a. | n.a. |
| Celluclast, buffer | 2.25 (0.07) | 1.08 (0.07) | n.a. | n.a. |
| Wastewater | n.a. | n.a. | 2.49 (0.63) | 0.98 (0.04) |
| CTec, wastewater | n.a. | 3.51 (0.04) | 2.72 (0.17) | 1.01 (0.19) |
| CTec, wastewater, EDTA | n.a. | 3.59 (0.24) | 1.97 (0.09) | 0.97 (0.03) |
| CTec, wastewater, 4× EDTA | n.a. | 3.41 (0.05) | 1.17 (0.05) | 0.55 (0.05) |
| Celluclast, wastewater | 2.38 (0.13) | 1.23 (0.25) | 2.24 (0.43) | 0.92 (0.08) |
| Celluclast, wastewater, EDTA | 2.34 (0.12) | 1.38 (0.11) | 2.25 (0.56) | 0.88 (0.05) |
| Celluclast, wastewater, 4× EDTA | 2.41 (0.15) | 1.34 (0.07) | 1.22 (0.34) | 0.57 (0.07) |

Numbers given in the parentheses are standard deviations, $n=3$

samples treated with EDTA (Table 4). EDTA may have cytotoxic effect on lactate-producing bacteria or affect the glucose to lactate metabolism or just removing produced lactate by complex binding lactate making it non-detectable by HPLC.

Samples with REnescience process wastewater contained lactic acid (lactate) and acetic acid (acetate) that can show useful in a biogas process but have an unfavourable effect on ethanol fermentation by yeast cells [7]. Not only is a high concentration of acetic acid inhibitory to yeast, but the contamination with lactic acid bacteria will be problematic for successful yeast fermentation for bioethanol production.

The high content of divalent ions such as calcium can have a profound effect on the flocculation of biomass polymers [25]. Dey et al. [25] found that biomass flocks are held together by cations and the removal of $Ca^{2+}$, $Mg^{2+}$ and $Fe^{3+}$ leads to disruption and release of proteins, carbohydrates and humic substances. Therefore, the utilisation of cation-binding agents, removing cations, could disintegrate the floc structure of pretreated MSW and eases the interaction between enzyme and substrate. These findings were not found in this study on model substrate using clean filter paper. However, due to the findings of high contents of divalent ions in this trial, future trials will have to measure the effect on floc structure of raw MSW material during enzymatic processing.

## Conclusion

Cellulases are the primary enzymes needed for hydrolysis and breakdown of organic fractions in unsorted MSW material. In this study, we found no effect of surfactants upon the enzymatic breakdown as measured by viscosity and particle size distribution. Concurrently, additional β-glucosidase to the cellulose preparations does not improve the viscosity in terms of lowering the viscosity in the slurry. MSW treatment wastewater was found to contain a high amount of calcium, potassium, sodium, chloride and others. Cellulase performance showed no effect of adding the metal ion-chelating agent EDTA to the solution wherein filter paper hydrolysis took place. Cellulases responded equally well when hydrolysing filter paper in buffer solution compared to the hydrolysation of filter paper in process wastewater. Cellulases were thus found to be robust in terms of degrading the organic fraction of MSW material.

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