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ENVIRONMENTAL BIOTECHNOLOGY

Simultaneous saccharification and fermentation of solid household waste following mild pretreatment using a mix of hydrolytic enzymes in combination with *Saccharomyces cerevisiae*

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Abstract Ethanol production from low severity pretreated (85 °C, 1 h) solid household waste was studied using simultaneous saccharification and fermentation (SSF). The aim of the study was to examine typical composition of the organic fraction of municipal solid waste (OFMSW) and to develop a simple method for simultaneous liquefaction and biofuels production. A model waste was prepared based on the composition of the organic waste in Masdar City. Chemical analysis of the OFMSW showed that it contained 37 % total solids with up to 57 g glucan/100 g total solid (TS). Hydrolysis of the wet OFMSW was carried out using a mix of hydrolytic enzymes: amylase, cellulase, protease, lipase, hemicellulase, and pectate lyase. The enzymatic hydrolysis using this enzyme mix was studied using different dilutions of the OFMSW at different enzyme loadings. This study has demonstrated that SSF of low severity pretreated OFMSW can be carried out using *Saccharomyces cerevisiae* without dilution (addition of water), and liquefaction of the undiluted OFMSW can be achieved in less than 24 h of hydrolysis. Also, SSF of the pretreated waste can be carried out with very low enzyme loading (10 % of the company recommended dosage)—0.1 % cellulase, 0.1 % amylase, 0.02 % protease, 0.02 % hemicellulase, 0.02 % lipase, and 0.02 % pectate lyase (*w*/*w* per TS) following mild heat pretreatment conditions of 85 °C for 1 h.

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Introduction

Generation and accumulation of waste is one of the world's fastest growing environmental problems. The increasing rate of solid waste generation is a result of increasing population, industrialization, and urbanization (Troschinetz and Mihelcic 2009; Zhang et al. 2010). In EU, 2.6 million tons of municipal solid waste is produced annually (Jensen et al. 2010), 212 million tons in 2006 for China (Zhang et al. 2010), and in the US, the number was 250 million tons per year in 2011 (Solid Waste and Emergency Response, EPA US 2013). In the Emirate of Abu Dhabi, up to 10 million tons of general waste (such as MSW, construction waste, electrical waste, medical waste, etc.) is generated annually at a daily rate of 1.8 kg of produced waste per person. Only about 4 % of the generated municipal solid waste (MSW) and agricultural waste is recycled, the rest is sent to landfill (The Center of Waste Management Abu Dhabi 2010; Statistics center Abu Dhabi 2011). The main problem with landfills is that landfills around the world are running out of space. Waste is being transported great distances from city areas to remote landfill (Curry and Pillay 2011). It is needless to say that this is not sustainable and contributes to GHG emission. The Emirate of Abu Dhabi has a goal of establishing a sustainable waste management system. The goal is to attain 80–90 % reduction by 2018 (The Center of Waste Mangement Abu Dhabi 2010).

Municipal solid waste should be considered a resource. The organic fraction contains carbohydrates—starch and some lignocellulose (Jayasinghe et al. 2011; Balat 2011;

Matsakas et al. 2014; Hussin et al. 2013), protein, lipid, and pectin (Jensen et al. 2010)—and has the potential to be used to obtain liquid biofuels (ethanol and biodiesel), biogas (methane and CO_2), syngas (hydrogen and CO), or pure hydrogen (Demirbas et al. 2011; Balat 2011; Demirbas 2010; Matsakas et al. 2014). In most countries, the organic fraction is the main component of MSW ranging from 38 % in Slovak to 51 % in Mexico (OECD Environmental Performance and Information Division 2007; Guadalupe et al. 2009). In Abu Dhabi, the organic fraction constitutes 73.7 % of the up to 1 million tons of MSW produced annually (Al Ashram 2008; Statistics center Abu Dhabi 2011). The use of organic waste for biofuel production could help tackle the two problems of alternative energy source and better waste management. Despite its availability in large quantities and possibility to decouple the food and biofuel production feud (Kaparaju et al. 2009), MSW is one of the least exploited biomass sources (Jensen et al. 2010). This could be attributed to the constraint of proper collection and segregation (Kretschmer et al. 2013) which increases the overall process costs. It has been suggested that segregation could be facilitated by liquefaction of the organic fraction by enzymatic hydrolysis (Jensen et al. 2010).

Hydrolytic enzymes convert complex carbohydrates to monomeric sugars (Ohgren et al. 2007) without formation of compounds that are inhibitory for fermenting organisms (Balat 2011; Jensen et al. 2010; Taherzadeh and Karimi 2007) which is the main advantage compared to processes that use chemical catalyst for hydrolysis. Hydrolysis aids the reduction of biomass viscosity via liquefaction which facilitates easy process mixing during fermentation (Jørgensen et al. 2006). Enzymatic hydrolysis is carried out at mild conditions (temperature 50 °C and pH 4.8–7) (Taherzadeh and Karimi 2007; Kim et al. 2011) reducing the process utility costs and corrosion problems as in the case of acid hydrolysis (Sun and Cheng 2002). Different enzymes are used because of the different components of organic fraction of municipal solid waste (OFMSW) (Jensen et al. 2010). A mixture of enzymes (amylase, cellulase, protease, lipase, hemicellulase, and pectate lyase) has been suggested for optimal hydrolysis of OFMSW (Novozymes A/S, Denmark).

This work investigates the possibility of liquefying OFMSW using the commercial Novozymes Biogas-Test Kit. We examine the lowest possible enzyme dosage for its liquefaction and hydrolysis after a mild severity pretreatment and the potential of this substrate as a feedstock for biofuel production. A model OFMSW was used to be able to achieve a relatively homogeneous substrate to allow for comparison of different process parameters.

Materials and methods

Raw material

MSW composition data was obtained from the waste collection company (LINC services) for 8 months (June 2011–Jan 2012). Based on the average composition of waste obtained, it was discovered that the highest fraction of MSW in Masdar City was the organic fraction (food and paper waste) as shown in Fig. 1. This is concurrent with the trend shown in different parts of the world and in Abu Dhabi (Al Ashram 2008; OECD Environmental Performance and Information Division 2007).

A model OFMSW (mOFMSW) was prepared based on waste data from Masdar City and composition reported in literature (OECD Environmental Performance and Information Division 2007). The samples were prepared using food products from the local markets mixed with paper waste in the ratio: 11 % rice, 5 % pasta, 7 % potatoes, 2 % corn, 4 % bread, 1 % pineapple, 1 % apple, 1 % carrot, 2 % cucumber, 3 % lemon, 1 % pawpaw, 6 % tomatoes, 3 % pickles, 6 % meat, 14 % fish, 6 % dairy, 2 % cabbage, 2 % lettuce, 2 % okra, 2 % eggplant, 2 % cauliflower, 2 % broccoli, 13 % vegetable oil, 1 % newspaper, 0.6 % cardboard, and 0.4 % A4 paper. To reduce the risk of spoilage, the mOFMSW was kept at 4 °C until use.

Solids and ash determination

Total solid and ash content was determined by drying the mOFMSW at 105 and 575 °C overnight, respectively.

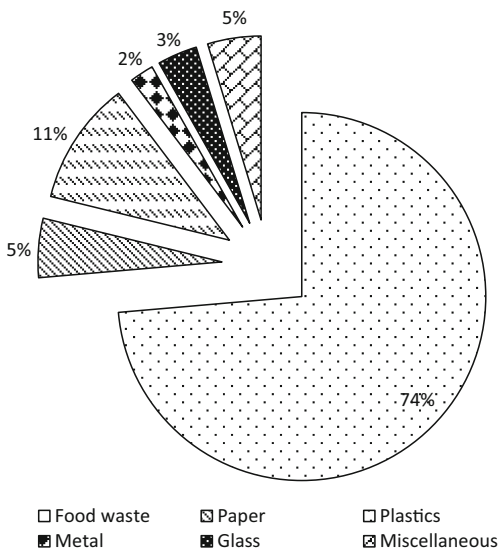

Fig. 1 Municipal solid waste composition from Masdar City, Abu Dhabi, UAE

Extractives determination

Extractives were removed prior to chemical characterization of the mOFMSW. This was carried out by subsequent water and ethanol extraction using Soxhlet apparatus water and ethanol extraction (Sluiter et al. 2008a, b). The dry biomass was grinded in a blender to ensure a homogenized sample for the HPLC analysis. The grinded dry biomass (5 g) was loaded into a cellulose thimble and the extraction was carried out with 200 g of the solvent for 12 h (per solvent used). Number of siphon cycles per hour was set to 3 for water extraction and 6 for ethanol extraction. Upon completion, the thimble contents were removed and dried. Water and ethanol extracts were analyzed for solids content by evaporating to dryness.

Water- and ethanol-soluble extractives (total and non-volatile) content in the raw biomass were calculated using Eqs. 1 and 2.

$$\text{Nonvolatile extractives(NE)}\left(\frac{\text{g}}{\text{100g TS}}\right)=\frac{W_{\text{dried water or ethanol extract}}}{\text{DM}}\times 100 \quad (1)$$

$W_{\text{dried water or ethanol extract}}$ Weight of the extract (evaporated to dryness) (g)
DM Dry matter of the raw sample (g)

$$\text{Total extractives (TE)}\left(\frac{\text{g}}{\text{100g TS}}\right)=\frac{\text{DM}-W_{\text{dried extracted biomass}}}{\text{DM}}\times 100 \quad (2)$$

$W_{\text{dried extracted biomass}}$ Weight of the extractives-free biomass removed from the thimble and dried (g)

Carbohydrate analysis of extractives-free solids

The extractives-free material was subjected to a strong acid hydrolysis according to Sluiter et al. (2008a). Up to 0.16 g of dried samples was treated with 72 % (*w*/*w*) sulfuric acid at 30 °C for 1 h and then the solutions were diluted with deionized water to achieve 4 % (*w*/*w*) of sulfuric acid concentration. Diluted samples were autoclaved at 121 °C for 1 h. The hydrolysate was filtered through fritted ceramic funnels, and the Klason lignin content was determined as the weight of the insoluble residue. The hydrolysate was analyzed for sugars using high performance liquid chromatography (Agilent 1260 Infinity Bio-inert Binary LC). The Hi Plex-H column (Agilent) and refractive index detector (RID) were used to determine the concentrations of glucose, xylose, and arabinose at 65 °C using 0.005 M H_2SO_4 as the mobile phase (eluent) with a flow rate of 0.6 mL/min.

Equations 3, 4, and 5 summarize the calculations made for the carbohydrates and Klason lignin content in the dry biomass.

$$\text{Sugar}_{\text{extractives-free}}\left(\frac{\text{g}}{\text{100g TS}}\right)=\frac{C_{\text{anhydro}}\times V_{\text{hydrolysate}}\times\frac{\text{1g}}{\text{1000mg}}}{\text{DM}_{\text{extractives-free}}}\times 100 \quad (3)$$

C_{anhydro} Concentration of the sugars converted into their polymeric form (glucose in form of glucan, etc.) using an anhydro correction (0.88 for pentoses and 0.90 for hexoses). Numbers were also corrected for any degradation that may have occurred during the dilute-acid step of the hydrolysis (using a recovery factor calculated from replicates spiked with known concentrations of the sugars analyzed) (g/L)
$V_{\text{hydrolysate}}$ Volume of the hydrolysate (mL)

The percentage of each sugar on an "as received" basis (including extractives) was calculated to demonstrate the true content of carbohydrates in the raw samples (Eq. 4).

$$\text{Sugar}_{\text{as received}}\left(\frac{\text{g}}{\text{100g TS}}\right)=\text{Sugar}_{\text{extractives free}}\times\frac{(100-\text{Extractives})}{100} \quad (4)$$

Acid insoluble lignin (Klason lignin) content in the extractives-free material was calculated using Eq. 5, while lignin content in the "as received" sample was calculated following the pattern of Eq. 4.

$$\text{AIL}_{\text{extractives free}}\left(\frac{\text{g}}{\text{100g TS}}\right)=\frac{(W_{\text{AIL}})}{\text{DM}_{\text{extractives free}}}\times 100\% \quad (5)$$

AIL Acid insoluble lignin (g/100 g total solid (TS))
W_{AIL} Weight of AIL after drying at 105 °C (g)

Starch content determination

Determination of starch in the raw biomass was determined by hydrolysis proceeded in two phases. Prior to hydrolysis the biomass was heated to 100 °C for 5 min. In phase I of hydrolysis, starch was partially hydrolyzed and totally solubilized by α-amylase (at 100 °C for 6 min). In phase II the starch dextrins were quantitatively hydrolyzed to D-glucose by

amyloglucosidase (at 50 °C for 30 min). Released glucose was measured by HPLC as described above.

Liquid carbohydrate analysis (of liquefied waste and fermentation broths)

Liquid fractions and fermentation broths were analyzed for the free sugars released including glucose, xylose, and arabinose, as well as for the fermentation products and by-products ethanol, acetic acid, and lactic acid. Approximately 3 mL of the liquid was centrifuged using a K3 series Centurion Scientific centrifuge at 2,000 rpm for 25 min. The supernatant was then filtered through a 0.2-μm filter into sampler vials for analysis on the HPLC. The analysis was performed using HPLC with the same operating conditions as described above.

Low severity pretreatment

Heat pretreatments at 85 °C for 1 h were carried out to pasteurize/sterilize the waste prior to SSF. Heat treatments were carried out in an oven in 1-L containers.

SSF

Simultaneous saccharification and fermentation (SSF) was carried out as described by Jensen et al. 2010 and Jørgensen et al. 2006 using baker's yeast (*Saccharomyces cerevisiae*, commercial dry yeast, Malteserkors tørgær, De Danske Spritfabrikker A/S, Denmark). SSF increases the overall process yield by reducing the inhibition effect of glucose on *S. cerevisiae* (Lin and Tanaka 2006; Kim et al. 2011). SSF of the heat-treated waste was carried out using 250-mL shake flasks equipped with yeast locks using two steps: pre-hydrolysis and fermentation.

The pretreated mOFMSW was cooled to 50 °C before adding the enzymes. On cooling, pre-hydrolysis was performed at 50 °C on the pretreated mOFMSW for a period of time depending on the operating condition being studied. Most of the experiments in this study were carried out using an enzymatic pre-hydrolysis time of 24 h. Enzymatic hydrolysis of the mOFMSW was carried out using commercial enzymes at constant loading (weight percent per total solids) in the ratio of cellulase complex:amylase:hemicellulase:pectate lyase:lipase:protease=1:1:1:0.2:0.2:0.2:0.2. This mixture will be further referred to as loading A. This ratio was selected based on the manufacturer's (Novozymes A/S, Denmark) recommended loading range.

After pre-hydrolysis, the hydrolysate was cooled down to 30 °C and inoculated with 0.2 g of *S. cerevisiae* for ethanol fermentation. The flasks were flushed with nitrogen gas to create anaerobic condition inside the shake flasks before it was fitted with a yeast lock filled with glycerol to keep anaerobic conditions. Fermentation was carried out for 3–5 days with a continuous agitation of 150 rpm in a shaking incubator. The flasks were weighted daily to measure weight loss and termination samples were extracted for HPLC analysis as described in "Carbohydrate analysis of extractives-free solids." No pH control was applied because we wanted to use as simple and cheap a process as possible.

Concentration of total ethanol produced was calculated based on the stoichiometric values and the mass of CO_2 produced per total solid (TS)—based on the flask weight loss (Eq. 6).

$$\text{Concentration of total ethanol produced}\left(\frac{\text{g EtOH}}{100\text{g TS}}\right) = \text{Concentration of } CO_2 \text{ produced}\left(\frac{\text{g } CO_2}{100\text{g TS}}\right) \times \text{stoichiometric ratio}\left(\frac{1.05\text{ g EtOH}}{\text{g } CO_2}\right) \quad (6)$$

The total concentration of ethanol produced was also obtained from HPLC analysis of the fermented broth.

Effect of substrate dilution and enzyme addition studied in shake flasks

The effect of substrate dilution was studied by using 30, 50, and 100 %*w*/*w* loading of the wet mOFMSW. For these screening experiments, pretreatment conditions were 85 °C for 1 h, pre-hydrolysis time of 24 h, and enzyme loading of 100 % A.

Effect of reduced enzyme loading studied in shake flasks

The effect of enzyme loading was performed on undiluted wet mOFMSW using 100 % A, 75 % A, 50 % A, 25 % A, 20 % A, 10 % A, 5 % A, and 0 % A (described in "SSF"). Conditions for these experiments were pretreatment of 85 °C for 1 h and pre-hydrolysis time of 24 h.

Analysis of changes in rheological properties during SSF

Rheological properties of the fermented broth were investigated by a stress-controlled ARES G2 Rheometer (TA instrument, Delaware, USA)—30-mm cup and bob concentric cylinder geometric configurations. This was to have an understanding on the interaction and nature of the solid aggregates in the suspension. The experiment was conducted using 5.48 mm of gap between bob and cylinder at a temperature of 20 °C using Advanced Peltier System (APS) temperature control with an accuracy of 0.1 °C.

Bioreactor experiments

SSF was carried out using 1,000 g of mOFMSW in a fermentor (Fermac 320, Electro lab Biotech Limited, UK) on heat-pretreated mOFMSW (85 °C for 1 h). Enzyme loading used for liquefaction and hydrolysis was 25 % A enzyme loading. The hydrolysate was corrected to a pH of about 5 (to be in the same pH range as experiments performed in the shake flasks) using 1 g of citric acid and 3.5 g of sodium carbonate after which it was inoculated with 1 g of yeast for simultaneous saccharification and fermentation (SSF). Mixing of the biomass was performed using standard CSTR impellers at 700 rpm in the bioreactor. No pH control was applied because we wanted to use as simple and cheap a process as possible.

Samples were drawn from the fermentor at intervals to measure the component concentration of the fermented broth on the HPLC. The experiments were carried out for around 60 h.

Results

Chemical composition of the model "organic fraction" MSW (mOFMSW) was examined by HPLC analysis after strong acid hydrolysis of polymeric sugars. The moisture content of the biomass was 63.12±1.52 %, while the total solid content of the mOFMSW was determined to be 36.88±1.52 %. The starch analysis showed that the dry mOFMSW contained 36.3 ±3.34 % starch. Extractives removal and strong acid hydrolysis showed 21.40±2.60 % glucan (primarily cell wall glucan), 1.35±0.17 % xylose, 0.18±0.05 % arabinose, 2.49±0.55 % lignin, 46.82±0.76 % extractives, and 4.03±0.03 % ash. As expected, the mOFMSW has low lignin content compared to more lignocellulosic types of waste. The mOFMSW is rich in glucan from starch and cellulose (from paper). The low lignin and ash content of the mOFMSW shows that the mOFMSW has a high biodegradability potential. The high content of water and ethanol extractives could be due to the high content of soluble sugars, proteins, and fats found in food waste. Analysis of the water extract is shown in Table 1. It can be seen that only a small amount of sugar monomers are present in the extracts; however, it is expected that this fraction contains a significant amount of starch.

Table 1 Compositional analysis of model OFMSW water extracts

	Concentration (g/100 g TS)
Glucose	1.57±0.00
Xylose	3.05±0.02
Lactic acid	1.33±0.01
Acetic acid	0.73±0.01

Effect of substrate dilution and enzyme addition studied in shake flasks

The solids content of the mOFMSW was found to be approximately 37 %, which means that 63 % of the waste is water. In future energy systems, water is a limited resource and the implementation of bioenergy processes, especially in arid regions, depends on the ability to minimize water usage. Enzymatic hydrolysis of the mOFMSW was examined using undiluted and diluted mOFMSW (Fig. 2). Reference experiments were performed without addition of enzymes. In Fig. 2, it can be seen that there was a significant increase, up to 50 %, in the ethanol produced when enzymes were used due to release of fermentable sugars as well as liquefaction of the substrate and hence more efficient stirring. Undiluted wet mOFMSW was liquefied in less than 24 h during enzymatic pre-hydrolysis but did not liquefy in the absence of enzymes (hence, ethanol could not be measured for this sample). Even though higher ethanol concentration was achieved with dilution of the MSW, efficient liquefaction was achieved during pre-hydrolysis in undiluted mOFMSW, and in order to

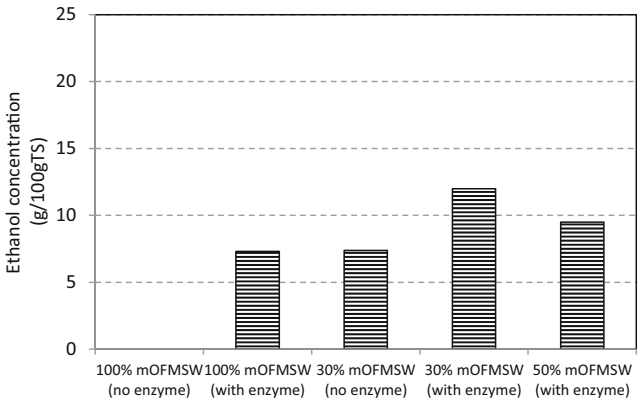

Fig. 2 Effect of wet mOFMSW dilution and enzyme addition on SSF. Results based on HPLC analysis of the fermented broth (100 g mOFMSW, 85 °C heat pretreatment for 1 h, and enzymatic hydrolysis using 100 % loading A at 50 °C for 24 h)

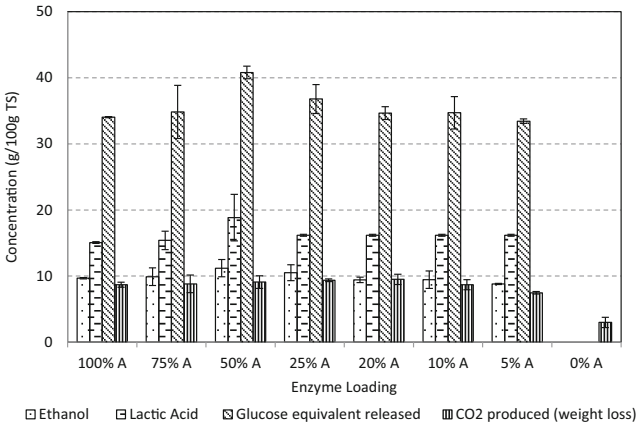

Fig. 3 Effect of enzyme loading on hydrolysis efficiency and concentration of ethanol produced after fermentation of wet mOFMSW (using 100 %OFMSW, heat pretreatment at 85 °C for 1 h, and 24 h pre-hydrolysis). Parameters shown are as follows: CO_2 from weight loss calculations and HPLC analysis of fermented broth, including measured ethanol and lactic acid concentration, and calculated equivalent glucose fermented

develop low input (water consuming) processes, it was decided to use undiluted mOFMSW (37 % TS) for subsequent experiments.

Effect of reduced enzyme loadings studied in shake flasks

Different enzyme loadings were tested using undiluted mOFMSW loading in SSF experiments. The obtained weight losses are shown in Fig. 3. The result suggests that at constant loading of 100 g wet mOFMSW, increasing the enzyme loading above 5–10 % A does not have a significant effect on process yield. Tukey's mean comparison based on the honestly significant difference confirmed those speculations, showing that no significant differences were detected among the results for different enzyme loadings. At enzyme dosage below 5 % A, the mOFMSW did not liquefy, and lower CO_2 liberation was observed (Fig. 3). For this sample, it was not possible to analyze on HPLC. Due to the difference between weight loss and measured ethanol obtained in the results shown in Fig. 2, ethanol and lactic acid was measured at the end of SSF; these results are also shown in Fig. 3. The remaining glucan and other sugars were fermented to lactic acid. The sugars released by enzymatic hydrolysis are shown in Fig. 3 as a glucose equivalent based on produced lactic acid and ethanol. Glucan equivalent fermented to lactic acid and ethanol was between 34 and 40 g/100 g TS corresponding to a significant fraction of glucan in the biomass (36 g starch/100 g TS and 21 g cellulose/100 g TS). Hence, very efficient hydrolysis was obtained in all experiments, showing that low enzyme dosage (5–10 % A) can be applied when using this process setup.

In order to further study the effect of the enzymes, rheological analysis of the broths was examined. The results shown in Fig. 4 showed a less pronounced shear thinning for samples of higher enzyme loading. The shear thinning of OFMSW samples with lower enzyme loading is higher—hence, it can be assumed that the rate of decrease of its high viscosity with increasing stress is faster (more pronounced). Since the samples with higher enzyme loading are already less viscous, increasing stress has little effect on its viscosity and, hence, the less pronounced shear thinning. These results indicate that the suspensions of higher enzyme loading are composed of suspended particles with lower degree of entanglement than the suspensions with lower enzyme loading. The observed decrease in the degree of entanglement at higher enzyme loading was a result of higher efficiency of the hydrolysis. Based on this, it can be suggested that there is better liquefaction of the mOFMSW with increasing enzyme

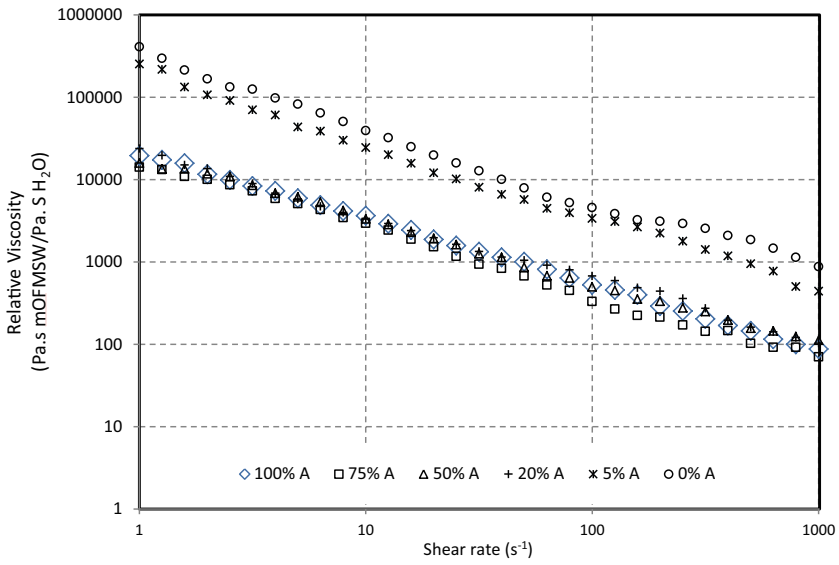

Fig. 4 Viscosity profile of fermented broth at different enzyme loadings using 100 %OFMSW, heat pretreatment at 85 °C for 1 h, and 24 h pre-hydrolysis

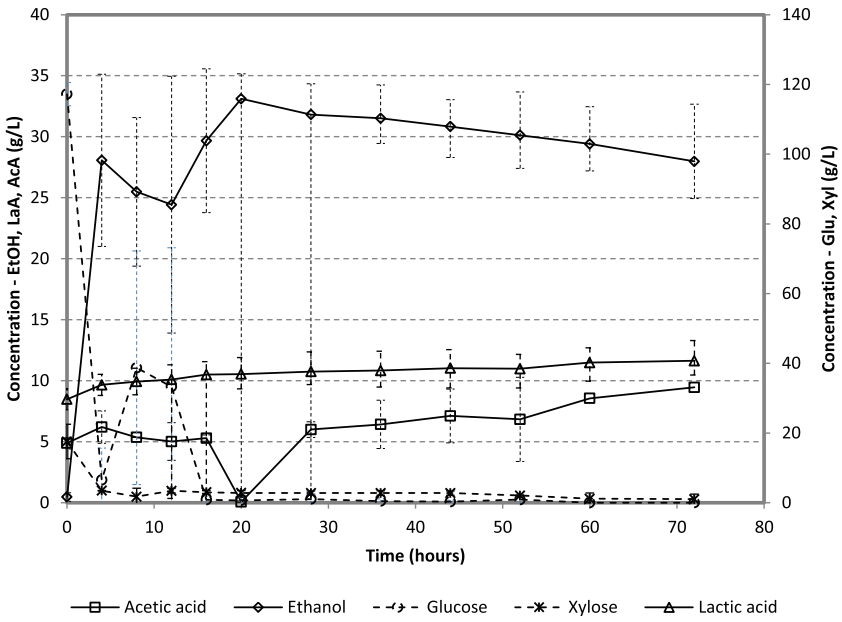

Fig. 5 Medium concentration change with time during fermentation in a laboratory-scale fermentor (1,000 g mOFMSW, enzyme loading 25 % A, and 85 °C heat pretreatment at 1 h.)

loading. However, even though Fig. 4 indicates better process liquefaction, it did not have a significant impact on the total ethanol yield after fermentation.

SSF in bioreactors

Based on the results of the batch (shake flaks) experiments, SSF was carried out in a laboratory fermentor with the possibility of sampling during the experiments (without disturbing the anaerobic environment) and more efficient stirring. These 1-L SSF experiments were carried out on undiluted MSW and enzyme loading of 25 % A (25 % A was chosen over 10 % because we wanted to avoid enzyme limitation at the less efficient stirring of the solid biomass in the bioreactor). Results of a duplicated experiment are shown in Fig. 5. The pH of the medium sampled at different intervals was in the range of 4.70–5.20. This is a low operating pH for the enzymes as this enzyme mix was designed to operate at pH close to neutral. However, glucose concentration is approximately 120 g/L at the time of inoculation, corresponding to approximately 50 % of glucan in the biomass. Furthermore, Fig. 5 shows that lactic acid concentration is already very high after hydrolysis at time zero, showing that lactic acid contamination takes place during the 24 h of pre-hydrolysis and that some of the hydrolysed glucose has already been converted. The inherent lactic acid concentration in the mOFMSW is

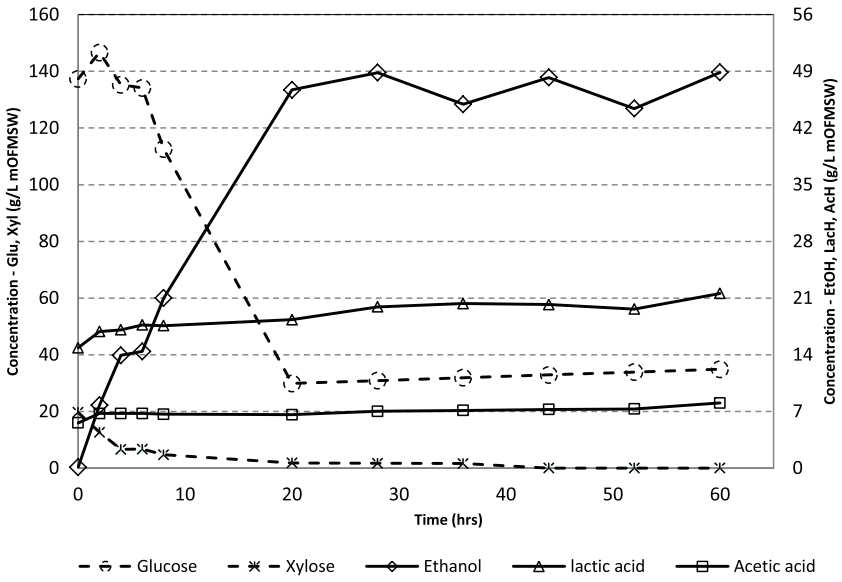

Fig. 6 Substrate consumption and product formation during fermentation—1,000 g mOFMSW, enzyme loading 25 % A, and 85 °C heat pretreatment for 1 h

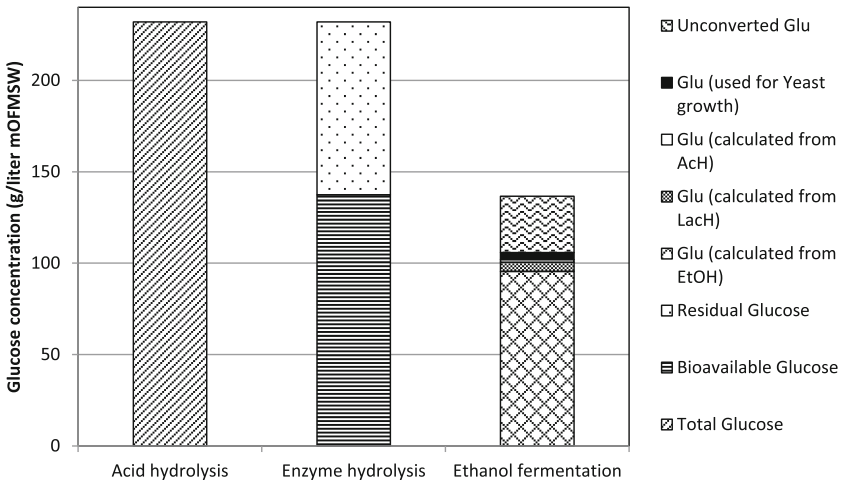

Fig. 7 Representation of overall SLFS hydrolysis and fermentation yields based on total glucose concentration of mOFMSW

approximately 5 g/L. However, after inoculation with *S. cerevisiae* at time zero, rapid ethanol production takes place. This shows that the inherent lactic acid bacteria do not inhibit ethanol production by the inoculated yeast.

Several SSF experiments were performed in this study. Results were somewhat inconsistent and showed high standard deviations, probably due to the very heterogeneous nature of this substrate. While many of the experiments performed resulted in lactic acid concentration in the range of 30–50 g/L, in one experiment, lactic acid formation was less abundant. This experiment is shown in Fig. 6. Very high productivity of 2.14 g ethanol/L×h was obtained in this experiment. The decline in xylose concentration seen in Fig. 6 may be connected to the increasing lactic acid concentration with time. After 20 h of fermentation, the ethanol production goes into stationary phase. Based on chemical characterization of the mOFMSW and results presented in Fig. 6, the process efficiency of enzymatic hydrolysis and fermentation was calculated. A graphical representation of the calculations is presented in Fig. 7. From Fig. 7, enzymatic hydrolysis yield was calculated as approximately 60 %, while ethanol fermentation yield based on the amount of bioavailable glucose for ethanol fermentation was approximately 70 %.

Effect of hydrolysis time studied in shake flasks

Based on observations in the bioreactor experiments, shake flask experiments were performed in order to examine if short hydrolysis time could reduce lactic acid contamination. The results in Fig. 8 show that longer hydrolysis time yields more bioavailable sugar—however, sugars are already released after 2 h of hydrolysis, and at this point, still no lactic acid is formed. These results indicate that 2-h hydrolysis should be

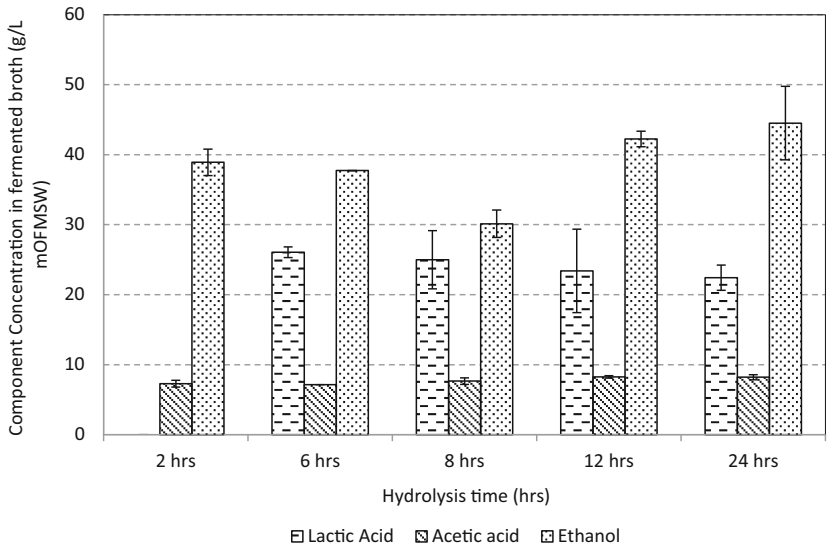

Fig. 8 Medium composition after fermentation of the mOFMSW using different enzymatic hydrolysis time. Data from HPLC analysis (100 g mOFMSW, enzyme mix 25 % A, and 85 °C heat pretreatment for 1 h)

applied in SSF of mOFMSW to reduce contamination with lactic acid bacteria during yeast fermentation of glucans.

Discussion

mOFMSW is a very heterogeneous substrate and it is solid at the beginning of the experiments which makes efficient stirring and process control difficult in initial stages of the process and, hence, repetition of experiments difficult. However, it is important to study and develop bio-process that can handle these complex substrates, especially in bioenergy processes where clean synthetic substrates are too expensive. The low lignin and high sugar content of the OFMSW make it a unique substrate for low temperature (low-energy penalty) biological processing because of the low recalcitrance compared to, e.g., pure lignocellulosic biomasses (Balat 2011). This study showed that the hydrolysis could be carried out without addition of fresh water. Fresh water is thought to become a limiting resource and minimizing fresh water consumption in future energy systems is important, even more so when developing biofuel systems in arid areas such as the Middle East. OFMSW contains 63 % water—some of which can actually be recycled as process water.

Results demonstrated the effect of different factors on hydrolysis of OFMSW based on the concentration of total glucose consumed. Liquefaction of undiluted mOFMSW (37 % TS) could be carried out successfully using a tailored enzyme mix (10 % of the company recommended dosage) in less than 24 h. High glucose concentration of 120–140 g/L liquefied mOFMSW was achieved in less than 24 h. This corresponds to approximately 60 % of glucan in the raw material based on starch determination by hydrolysis and cellulose determination by extraction followed by strong acid hydrolysis. However, there might be a slight overestimation of glucan in the raw material if not all starch is removed by extraction prior to cellulose determination. Of the bioavailable glucose, 70–90 % was consumed by the yeast for ethanol production in 24 h of fermentation, and residual glucose and pentose sugars were fermented to lactic acid. The overall SSF yield of around 50 % based on the bioreactor experiment can be increased with better pH and contamination control. Optimal pH of the enzyme mix was in the range of pH 6–7; however, toward end of the fermentation pH was 5 or below. Even though the aim of this study was a low cost process without pH control, this might be applied in future studies. Weiss et al. (2013) showed that cellulose hydrolysis is inhibited at glucose concentrations above 10 g/L. In Fig. 6, the concentration of glucose at time zero was up to 140 g/L wet mOFMSW. Therefore, incomplete hydrolysis of mOFMSW can be due to a combined inhibition effect of low pH, lactic acid concentration (due to possible process contamination), and high glucose concentration.

The difference between weight losses and measured ethanol concentration found in these experiments as well as analysis of the metabolic products showed contamination, e.g., with lactic acid bacteria and possibly other microorganisms. These microorganisms have the ability to convert some components of the substrate to other by-products, releasing CO_2 in the process (Demirbas et al. 2011; Abdel-Rahman et al. 2011). Furthermore, anaerobic conditions might not have been initially present in the shake flasks, giving CO_2 liberation from biomass growth without ethanol production. John et al. 2009 stated that the enzymatic action of cellulose hydrolysis is reduced with an increase in lactic acid concentration. According to Jacques et al. 2003, concentrations of 0.8 %*w*/*v* of lactic acid inhibit the action of yeast during fermentation—this is lower than the concentration of lactic acid in the experiments shown in Fig. 5. Notwithstanding the initial significant concentration of lactic acid, ethanol is still being produced with a yield of approx. 70 % (on available glucose). Combining ethanol and lactic acid production shows that 113 g/L of glucose equivalent sugar has been consumed, which is close to the 120 g/L measured at time zero. Very high productivity of 2.14 g ethanol/L×h was obtained in the experiment where no contamination was observed (Fig. 6). For comparison, 1.5 g ethanol/L×h was reported by Ohgren et al. 2007 in pretreated corn stover substrate. Xylose is also consumed in this experiment showing co-fermentation of yeast and lactic acid bacteria, as some strains of *Lactobacillus* have the capacity to utilize pentose sugars such as xylose for lactic acid production (Cui et al. 2011; Abdel-Rahman et al. 2011).

The main result from this study is the high potential of the organic fraction of municipal solid waste to be used as nutrient and glucose rich (120–140 g/L) substrate for microbial fermentation using no fresh water and very low enzyme dosage. OFMSW has an advantage over lignocellulosic substrates because it does not contain fermentation inhibitors (such as HMF, furfural, and phenolics) produced by high temperature physico-chemical pretreatment of lignocellulose prior to enzymatic hydrolysis. This makes OFMSW suitable for bacterial as well as yeast fermentation. However, contamination with unwanted microorganisms needs to be controlled.

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