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Ethanol Production from the Organic Fraction Obtained After Thermal Pretreatment of Municipal Solid Waste

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Abstract In this work, the use of organic fraction from municipal solid waste (MSW) as substrate for ethanol production based on enzymatic hydrolysis was evaluated. MSW was subjected to a thermal pretreatment (active hygienization) at 160 °C from 5 to 50 min. The organic fiber obtained after 30 min was used as substrate in a simultaneous saccharification and fermentation (SSF) and fed-batch SSF process using cellulases and amylases. In a fed-batch mode with 25% (*w*/*w*) substrate loading, final ethanol concentration of 30 g/L was achieved (60% of theoretical). In these conditions, more than 160 L of ethanol per ton of dry matter could be produced from the organic fraction of MSW.

Keywords Municipal solid waste · Thermal pretreatment · Second generation bioethanol · Enzymatic hydrolysis · Simultaneous saccharification and fermentation · Fed-batch SSF

Introduction

The demand for bioethanol is increasing worldwide. It has been estimated that ethanol production rate by 2050 will be 50 times larger than the ethanol production of 2004 [1]. Such a large production of ethanol will require reliable sources of feedstock. Nowadays, bioethanol, the most important renewable fuel in terms of volume and market value, is mainly produced from sugar- and starch-based materials such as sugar cane and corn [2]. However, present methods of producing biofuels have been criticized for causing high food prices by taking up land that would have been used to grow food and deforestation [3]. Thus identifying alternative sources of biofuel feedstock should be a high priority. In this context, ethanol produced from lignocellulosic feedstocks such as forest residues, grasses, and organic fractions of municipal solid waste (MSW) is emerging as an attractive option

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because of lower feedstock costs and higher potential for fossil fuel displacement and reduction in greenhouse gas emissions compared with corn-ethanol [4]. Although many lignocellulosic materials such as wheat straw [5], barley straw [6], corn stover [7], spruce [8], etc. have been investigated as potential feedstock for ethanol production, little research has been done on MSW. This material is an especially appealing feedstock for ethanol production, because cellulosic materials such as paper, wood, and yard waste form about 60% of the dry weight of a typical MSW [9]. Food waste, especially vegetables and fruits, are also an important source of biosolids that could be used for ethanol production [1]. Apart from this, the use of the organic fraction of MSW for bioethanol production would reduce the amount of MSW going to landfill which is one of the requirements of EU Landfill Directives [10, 11]. Considering the large amount of solid waste that is being generated in the world, especially in developed countries, a judicious management of MSW would not only help to address the problem of MSW disposal but also contribute toward diversifying feedstock sources for bioethanol production [1].

At present, two processes to convert MSW into biofuels are being developed at industrial scale using different production pathways such specifically thermo-chemical [12, 13] and biochemical [14]. Conversions in the thermo-chemical way, using heat, pressure, and steam, MSW are converted into synthesis gas (syngas), and subsequently, the cleaned gas is passed through a catalyst and converted into liquid biofuels, such as ethanol, as well as chemicals. In the biochemical way, the cellulosic fraction of the feedstock is hydrolyzed using concentrated acid, and then the remaining acid–sugar solution is separated into its acid and sugar components by means of chromatographic separation, and sugars are fermented to ethanol.

One biochemical alternative to acid hydrolysis of lignocellulose could be enzymatic hydrolysis. However, due to the heterogeneous nature of the organic fraction of MSW, the enzymatic conversion route has not been well investigated. In this work, the use of organic fraction of MSW as substrate for ethanol production based on enzymatic hydrolysis was evaluated. For that, MSW was subjected to a thermal pretreatment (active hygienization) in which the residues were exposed to different times at high temperature and pressure. Then, after active hygienization, the pretreated material was separated using conventional separation equipment in different fractions such as plastic, metals, glasses, and the organic fiber. As well as obtaining a material that could be a suitable substrate for ethanol production, other advantages of this pretreatment are no unpleasant smell is produced, waste volume reduction of up to 80%, and absence of pathogenic agents [15]. The organic fiber obtained was used as substrate for ethanol production by simultaneous saccharification and fermentation (SSF) and fed-batch SSF using different cellulase loadings. Finally, the potential ethanol that could be produced from this substrate was calculated.

Materials and Methods

Raw Material

The organic fraction of MSW obtained after a thermal pretreatment (organic fiber hereinafter) was supplied by Ambiensys S.A.

Pretreatment

MSW (as collected from traditional waste containers) was subjected at a thermal pretreatment called “active hygienization.” The pretreatment was carried out by Ambiensys

S. A., in batch equipment with 1,000-kg capacity in an environment of steam at 160 °C. The effect of different residence times (from 5 to 50 min) on organic fiber composition was evaluated.

After pretreatment, the hygienizated material was separated in different fractions (organic fiber, plastic, metal, glass, etc.) by using a trommel screen with 10-mm mesh openings, and a small amount of impurities (about 3%), mainly glasses, still remain in the organic fraction. Then, the organic fiber obtained after pretreatment was packed in 2-kg bags and frozen until use.

Enzymatic Hydrolysis

Enzymatic hydrolysis (EH) tests were carried out in 250-mL Erlenmeyer flasks containing 100 g of medium at pH 5.0 with different organic fiber concentrations (10% and 20% *w*/*w*) at 50 °C for 72 h and 150 rpm in an orbital shaker. Samples were withdrawn at 24, 48, and 72 h and centrifuged for 10 min at 12,500 rpm, and the supernatant was analyzed for glucose. The effect of enzyme loading (20, 40, and 60 FPU/g of cellulose and ß-glucosidase 20, 40, and 60 IU/g cellulose) and substrate concentration (10% and 20% *w*/*w*) on glucose production was tested. The activity of the two enzymes cellulase (NS50013) and ß-glucosidase (NS50010) were determined to be 60 FPU/mL and 700 IU/mL, respectively, according to methods described by Ghose [16]. Both enzymes were kindly supplied by Novozymes A/S (Bagsværd, Denmark).

Previous to EH experiments, the organic fiber was autoclaved at 121 °C for 20 min in order to avoid any kind of contamination.

Microorganism

Saccharomyces cerevisiae (Fermentis Ethanol Red, Marcq en Baroeul Cedex, France) was used in this work.

Simultaneous Saccharification and Fermentation

The organic fiber obtained after active hygienization at 160 °C for 30 min supplemented with 2 g/L yeast extract, 2 g/L NH_4Cl, 1 g/L KH_2PO_4, and 0.3 g/L $MgSO_4 \cdot 7H_2O$ was used as substrate for batch and fed-batch SSF processes. SSF experiments were carried out in an orbital shaker at 32 °C for 72 h and 150 rpm in 250-mL Erlenmeyer flask each containing 100 mL of medium at an initial pH 5.0. Batch SSF was carried out with 20% (*w*/*w*) substrate concentration. Fed-batch SSF experiments started with 20% (*w*/*w*) substrate loading, and at 24 h from the onset, a batch of 5% (*w*/*w*) was added. Fed-batch SSF runs were carried out for 96 h. In order to maintain a constant enzyme to substrate ratio, extra enzyme was added together with extra substrate. Different enzymes loading (20–60 FPU/g of cellulose) of NS50013 and (20–60 IU/g cellulose) of NS50010 were employed. In some experiments prior to SSF, a liquefaction and presaccharification steps were carried out using Termamyl (0.05% *w*/*w*) and Spirizyme Fuel (0.1% *w*/*w*) at 85 °C for 1 h and at 60 °C for 3 h, respectively. All enzymes were kindly supplied by Novozymes A/S (Bagsværd, Denmark). All SSF flasks were inoculated with 1 g/L of dried yeasts added directly.

Previous to SSF experiments, the organic fiber was autoclaved at 121 °C for 20 min in order to avoid any kind of contamination.

Analytical Methods

The organic fiber obtained after active hygienization of MSW at different times of pretreatment was analyzed using the National Renewable Energy Laboratory standard methods for determination of structural carbohydrates in biomass [17].

Glucose and xylose concentration were determined by high-performance liquid chromatography in a Waters 2695 liquid chromatograph with refractive index detector. A CARBOSep CHO-682 LEAD column (Transgenomic, Omaha, NE, USA) operating at 80 °C with Milli-Q water (Millipore) as mobile-phase (0.5 mL/min) was used.

Ethanol was analyzed by gas chromatography, using a HP 5890 Series II apparatus equipped with an Agilent 6890 series injector, a flame ionization detector, and a column of Carbowax 20M at 85 °C. Injector and detector temperature was maintained at 150 °C.

Results and Discussion

Effect of Pretreatment Time on the Organic Fiber Composition

About 1,000 kg of MSW as collected from waste containers was used in each pretreatment condition. Organic fiber (moisture content about 50%) recovery was in the range of 20–25% (dry weight). Table 1 shows the composition of the organic fiber obtained after different pretreatment times of MSW. Glucose was the main component followed by acid insoluble residue and ash. As can be seen, longer residence time slightly increases the glucose content in the fiber, varying from 37.5% when the residence time was 5 min to 43.9% when the MSW was pretreated for 50 min. This increase of glucose fraction after pretreatment could be due to solubilization of other compounds (such as fats, oils, fatty acids, etc.) that were not analyzed. Regarding xylose content, it was about 5% for all times tested. This increase in glucose content without a xylose decrease when longer pretreatment times were used suggests that no sugar degradation was produced during active hygienization. That means that total carbohydrate content of the organic fiber was about 47–49%, which is still low when compared to other lignocellulosic materials whose sugar content is over 70% [5–9]. Nevertheless, taking into account the nature of this feedstock and its negative cost, the use of the organic fraction of MSW as substrate could be considered as an interesting alternative for ethanol production. On the other hand, insoluble acid residue and ash were in the range of 22–29% and 14–18%, respectively. Moisture content of the organic fiber was in the range of 52–55%.

Table 1 Composition (% of dry matter) of the organic fiber obtained after active hygienization at differents times.

Treatment conditions	Glucose (%)	Xylose (%)	Insoluble acid residue (%)	Ash (%)	Others (%)
160 °C; 5 min	37.5±1.6	5.8±0.2	24.6±1.1	18.0±0.9	14.1
160 °C; 10 min	37.6±1.5	5.0±0.3	29.1±1.0	17.9±1.1	10.4
160 °C; 20 min	40.2±1.8	5.0±0.3	21.9±0.8	17.7±0.7	15.2
160 °C; 30 min	41.9±1.7	5.2±0.2	22.6±0.9	13.9±1.1	16.4
160 °C; 50 min	43.9±1.7	5.5±0.2	23.1±1.1	14.3±1.2	13.2

Data as mean of three replicates plus standard deviation

Considering the highest glucose and xylose content of the organic fiber and in order to save energy during pretreatment, fibers obtained after an intermediate time of pretreatment (30 min) was chosen as material for EH and SSF tests.

Enzymatic Hydrolysis

Figure 1 shows the effect of enzyme loading at two substrate concentration (10% and 20% *w*/*w*) on glucose production when using the organic fiber obtained at 160 °C and 30 min. Glucose concentrations found in different runs corresponded to enzymatic hydrolysis yield in the range of 37–44% of theoretical (based on potential glucose content in the organic fiber) for both substrates concentrations. As can be seen, the lowest glucose concentration (30 g/L) was reached at the lower enzyme loading employed (20 FPU/g cellulose) when substrate concentration was 20% (*w*/*w*). Nevertheless, no significant differences were observed at enzymes loading of 40 and 60 FPU/g cellulose, and similar glucose concentrations (34.8 and 36 g/L, respectively) were obtained. In both cases, although maximum glucose concentration was reached at 72 h, hydrolysis was almost completed at 48 h. However, at lower enzyme loading (20 FPU/g cellulose), significant differences regarding glucose released could be observed between 48 and 72 h (26 and 30 g/L of glucose, respectively). Nevertheless, the results seem to indicate that, when using 20 FPU/g cellulose, higher amount of glucose could be reached if longer incubation times had been used.

On the other hand, when 10% (*w*/*w*) substrate concentration was used, maximum glucose was reached after 48 h of hydrolysis in all cases studied. When enzymes loading of 40 and 60 FPU were used, similar glucose concentrations were achieved (about 19 g/L), and no significant differences were observed.

Other authors [9] obtained slightly higher glucose yield (about 60% of theoretical) in a work with autoclaved MSW at similar enzymes loading. However, it should be noticed that in that study, the organic fraction was pretreated with dilute sulfuric acid at 165 °C, and lower substrates concentrations (equivalent to 2% cellulose) were used during the

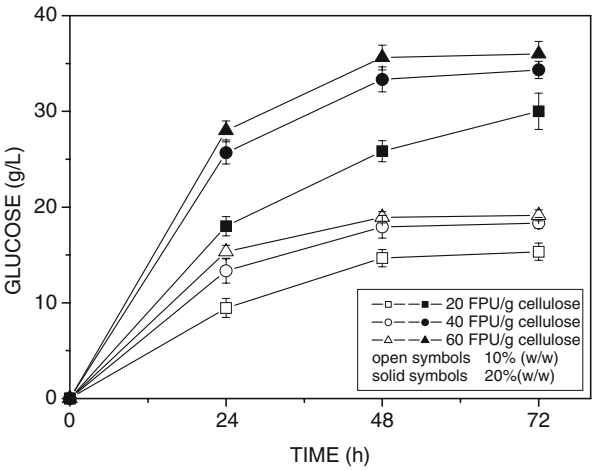

Fig. 1 Effect of substrate concentration (10% open symbols; 20% solid symbols) and enzyme loading on glucose production using the organic fiber obtained from MSW pretreated at 160 °C for 30 min. Data as mean of three replicates (*bars* represent standard deviation)

enzymatic hydrolysis. Li et al. [11] reached higher enzymatic hydrolysis yields (about 90%) when using selected biodegradable waste (carrot peelings and potatoes peelings, grass, and newspaper) after dilute acid hydrolysis followed by steam pretreatment. In that study, the authors concluded that the effect of enzyme loading was significantly high, so optimal conditions were obtained at higher enzyme loadings (60 FPU/g substrate) and low substrate concentrations. Similar results (around 90% glucose yield) were obtained by Nguyen et al. [18] with different biomass material chosen to simulate the lignocellulosic component of MSW (fir, almond tree pruning, wheat straw, and waste paper) subjected to acid impregnation and steam pretreatment and subsequent enzymatic hydrolysis at high enzyme loadings (66 FPU/g cellulose). It is noteworthy that in both cases, the material used tried to simulate the organic fraction of MSW, and high enzymes loading were employed. The differences found with the present work might be due to that in this study the organic fraction of MSW as collected from traditional waste containers was used as substrate, no acid was employed during the pretreatment, and lower enzyme loadings were used.

Simultaneous Saccharification and Fermentation

As was mentioned above, the organic fiber obtained after thermal pretreatment for 30 min was chosen for SSF test. According to the results obtained during EH tests in which it was shown that similar glucose yields were reached with both substrates concentrations, only the highest substrate concentration (20% *w*/*w*) was used for SSF tests with the aim of producing as high ethanol as possible. Figure 2 shows the effect of different enzymes loading on ethanol production during SSF using the pretreated organic fiber. As can be seen, differences in ethanol concentration can only be observed at 24 h of process where about 17 g/L of ethanol was produced with the lower enzyme loading employed (20 FPU), while ethanol concentrations of about 21 g/L were reached when 40 and 60 FPU were used. At 48 h, similar final ethanol concentration was obtained for all enzymes loading tested (about 24–25 g/L). No further increase in ethanol concentration was found when fermentation was extended to 72 h. That means that the highest volumetric productivity was reached at 24 h (0.7 $gL^{-1}h^{-1}$). Glucose concentration determined in the fermentation

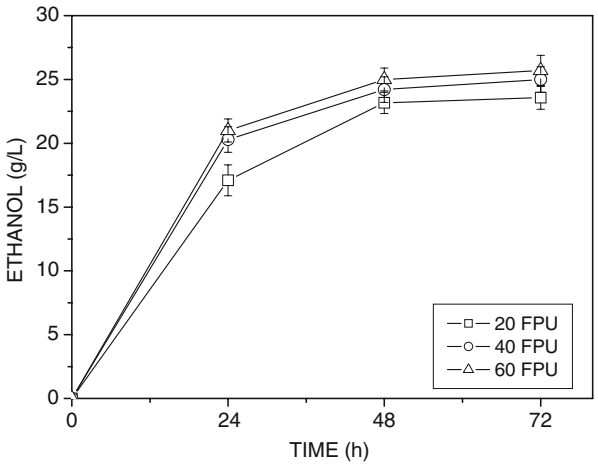

Fig. 2 Time course of SSF with 20% (*w*/*w*) organic fiber obtained at 160 °C for 30 min at different enzymes loading. Data as mean of three replicates (*bars* represent standard deviation)

broth was lower than 0.2 g/L in all the samples withdrawn throughout the fermentation runs. Since *S. cerevisiae* is not capable of fermenting pentoses, xylose concentration remained constant (about 2 g/L) in the fermentation broth during the SSF process (data not shown).

As was shown in the EH tests (Fig. 1), the use of higher enzymes loading resulted in higher glucose concentrations. This difference was not found in SSF experiments, which means that the use of lower enzyme loadings (20 FPU/g cellulose) is favored in an SSF process, attaining the same ethanol yields than those obtained with higher enzymes loadings. This positive effect of an SSF process regarding the use of lower enzymes dosages has been proved previously with other lignocellulosic substrates [19, 20].

The ethanol concentrations reached by an SSF process (24–25 g/L) on the selected pretreated organic fiber at initial substrate loading of 20% (*w*/*w*) mean an ethanol yield about 60% of theoretical.

Taking into account that the use of higher enzymes (cellulases) loading did not result in higher ethanol yield and according to the heterogeneous nature of the material used in which some amount of starchy material could be present, the next step in this work was to supplement the SSF process with alpha and glucoamylases in an attempt to increase the glucose released and, therefore, the ethanol concentration. Results of the effect of amylases addition to an SSF process together with a cellulase loading of 20 FPU/g cellulose are shown in Fig. 3.

As can be seen, the addition of both alpha and glucoamylases did not result in a higher amount of ethanol in the fermentation broth when compared to an SSF without amylases addition. Similar final ethanol concentrations (about 25 g/L) were produced in both cases. Contrary to this results, in a work using kitchen garbage as substrate for ethanol production by an SSF process, the addition of amylases resulted in relative high ethanol yield due to the high starch content of kitchen garbage [21]. However, our results suggest either the starch content of the organic fraction was very low or the amount of amylases used was not enough. Considering that the amylases loading employed in this work was in the range of those used in distilleries [22], the results obtained seem to indicate that the starch content of the organic fraction of MSW used in this work was not as high as expected. Other possible

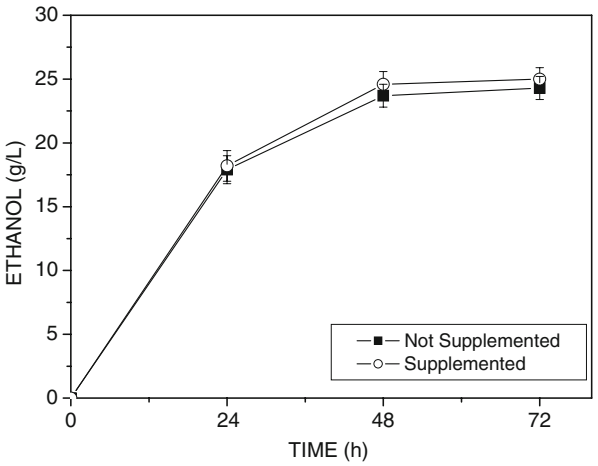

Fig. 3 Effect of amylases addition on ethanol production by SSF process with 20% (*w*/*w*) organic fiber obtained at 160 °C for 30 min. Data as mean of three replicates (*bars* represent standard deviation)

explanation is that starch was solubilized during the thermal pretreatment. Nevertheless, a complete characterization of the material should be done in order to know the exact nature of this material.

In an ethanol production process, the final concentration should be as high as possible in order to reach at least an ethanol concentration of 4% (*w*/*v*), which is considered as benchmark for reducing distillation [23]. Final ethanol concentration is directly related with substrate loading in the SSF broth. When using lignocellulosic materials as substrates for SSF process, substrates loading of about 14–15% (*w*/*v*) would be needed to reach such concentration [24]. In this work, using batch SSF process with an initial substrate loading of 20% (*w*/*w*), the ethanol concentration achieved was still below this benchmark. Therefore, with the objective of increasing the ethanol concentrations, batch SSF with higher substrate loadings (25% *w*/*w*) were performed (data not shown). However, due to the high viscosity of this medium, the experiments did not run properly. So, a fed-Batch SSF starting with 20% and a 5% (*w*/*w*) substrate addition at 24 h as described in "Materials and Methods" was studied. Ethanol concentrations reached during fed-batch SSF are shown in Fig. 4.

As can be seen, fed-batch SSF followed a similar pattern to batch SSF, reaching the highest ethanol concentration (about 30 g/L) at 72 h of process. So, this process configuration resulted in higher ethanol concentrations in comparison to those reached in batch mode (about 25 g/L) that are close to that considered as a benchmark.

Although the ethanol yield obtained in this work is still a little lower in comparison to those obtained with other lignocellulosic substrates, it should be kept in mind the complex nature of this substrate. On the other hand, it should be noticed that the pretreatment used in this work was originally focused on separating the different fractions that MSW are made of and reducing the amount of waste to be sent to landfill. Therefore, in future work, pretreatment conditions could be optimized in order to obtain an organic fiber that shows a higher accessibility to enzymatic hydrolysis and much more suitable to be used as feedstock for ethanol production.

Based on these results and using the organic fiber obtained after active hygienization of MSW at 160 °C for 30 min as substrate in a fed-batch process more than 160 L of ethanol per ton of dry matter could be produced from the organic fraction of MSW.

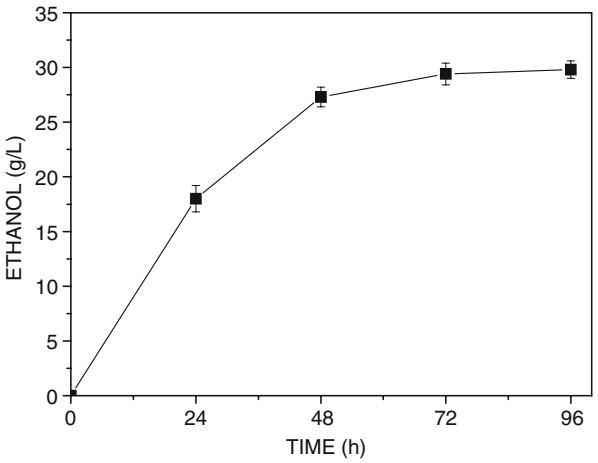

Fig. 4 Time course of fed-batch SSF. Data as mean of three replicates (*bars* represent standard deviation)

Concluding Remarks

Thermal pretreatment (active hygienization) seems to be an adequate method to obtain an organic fraction from MSW collected from waste containers suitable for ethanol production. At the temperature used in this work, longer residence times slightly increase sugar content and no sugars degradation was observed. Using a fed-batch process configuration with the organic fiber obtained after 30 min of treatment, ethanol concentrations about 30 g/L, close to that considered as benchmark for distillation process could be obtained. Considering that the material used as substrate was obtain from MSW, the ethanol yield reached (about 60% of theoretical) makes this material an attractive feedstock for second generation ethanol production.

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