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Municipal Solid Waste as Carbon and Energy Source for *Escherichia coli*

Erica Rosander, Maria Svedendahl Humble* and Andres Veide*

KTH Royal Institute of Technology, School of Biotechnology, Division of Industrial Biotechnology, AlbaNova University Center, Sweden

Abstract

Municipal solid waste (MSW) is an attractive renewable carbohydrate resource. MSW has a naturally high carbohydrate content that can be utilized as a carbon and energy source for bacterial cultivation. There is industrial interest in implementing MSW as a sustainable cultivation medium for biological production in biorefinery platforms. The present study investigated the potential of MSW as a carbon and energy source for *Escherichia coli*. For this purpose, MSW was initially fractionated and processed to yield a liquid (LMSW) and a dry solid fraction (DSMSW). In contrast to DSMSW, the liquid fraction had a natural high sugar content of 21 g L^{-1}. By further applying enzyme-catalyzed hydrolysis to DSMSW, a hydrolyzate concentration of 114 g L^{-1} was achieved. The two MSW fractions were evaluated separately as carbon and energy sources for *E. coli* in batch and fed-batch cultivations.

Keywords: Biorefinery; Enzyme hydrolysis; Household food waste; Lignocellulose; Waste valorization

Introduction

Municipal solid waste (MSW) is abundant. In the year 2013 alone, more than 1 billion tonnes of MSW were produced worldwide [1]. To find alternative managing methods as well as methods for further valorization and utilization of MSW have become vital. The definition of MSW is broad. It is normally defined as any waste product discarded by the public. In Europe, the average organic fraction of MSW (OFMSW) is 35 % [2].

OFMSW is rich in carbohydrates with glucans (29.8 *w/w* %) and xylans (20.9 *w/w* %) as the main components [3]. Since waste is not suitable for human consumption, other applications could be considered for this resource. For example, MSW may be applied as a biorefinery feedstock.

MSW contains inaccessible amounts of sugars locked in carbohydrate polymeric structures. In order to enable the utilization of MSW as a substrate for microorganisms, the inaccessible amounts of sugars must be degraded into monomeric sugars. For carbohydrate degradation, acid- or enzyme-catalyzed hydrolysis can be applied. Acid-catalyzed hydrolysis is effective. However, milder hydrolysis reaction conditions are preferred to prepare a bacterial cultivation medium. Enzyme-catalyzed hydrolysis can be applied in mild reaction conditions, and no neutralization of the final hydrolyzate is required. Hydrolysis of MSW will result in different monomeric sugars, since it normally contains both glucans and xylans.

There are several challenges associated with simultaneous sugar uptake in *Escherichia coli* [4,5]. However, the full potential of MSW as a carbon source will not be reached without effective and simultaneous assimilation of various sugars. Recently, an adopted *E. coli* strain, named PPA652ara, was developed to simultaneously take up D-glucose, D-xylose, and L-arabinose [6]. In this strain, the ptsG gene is deleted [7]. Consequently, the normal mechanism for glucose assimilation, regulated by the protein $EIICB^{Glc}$ is removed. Thus, carbon catabolite repression is relieved, and the genes needed for the uptake of secondary carbon sources is transcribed. This in-house generated *E. coli* strain was applied in this study.

E. coli is an excellent organism for the biological production of bio-based chemicals. This is mainly due to its low production costs, its rapid cell growth, our extensive knowledge of its metabolic pathways, and the available tools for genetic engineering [8]. In addition, *E. coli* has high biosynthetic capacity and can grow well in a defined mineral medium supplemented with a carbon source. Energy can be saved if the cell growth rate is increased. This can be achieved by using a complex and rich medium containing carbon precursors, which can immediately enter anabolic pathways after cellular assimilation. However, a complex medium may also contain compounds inhibiting cell growth and/or product formation [9]. MSW has a complex character, and a full characterization of all ingoing components would be costly. The development of a MSW-driven biorefinery based on *E. coli* requires that the effects of MSW on *E. coli* growth are explored. Previous studies have not discussed the complexity of the substrate and its effects on cellular metabolism. In most cases, cellular assimilation of only one type of sugar has been evaluated.

To fill the gap between MSW hydrolysis and its effect on cellular growth, an evaluation of MSW fractionation, the hydrolysis of different fractions, and the subsequent influence on cellular growth, is required. Also, the potential of MSW as a lignocellulosic carbon source for cell growth would increase if an *E. coli* strain able to assimilate more than one type of sugar were applied.

In this study, MSW was fractionated into a liquid, and a solid fraction. The properties and the full potential of both MSW fractions as a carbon and energy source for *E. coli* were carefully investigated. Finally, this approach to evaluating both the hydrolyzed liquid and solid fractions of MSW as a nutrient source for *E. coli* in fed-batch mode for the cellular assimilation of more than one sugar is novel.

Materials and Methods

Chemicals

Concentrated sulfuric acid (97 %), sodium diacetate, and disodium acetate were purchased from Merck Millipore. Dilute sulfuric acid (0.1

***Corresponding authors:** Maria Svedendahl Humble, KTH Royal Institute of Technology, School of Biotechnology, Division of Industrial Biotechnology, AlbaNova University Center, Stockholm SE-106 91, Sweden, Tel: +4687909944; E-mail: mariasve@kth.se

Andres Veide, KTH Royal Institute of Technology, School of Biotechnology, Division of Industrial Biotechnology, AlbaNova University Center, Stockholm SE-106 91, Sweden, Tel: +4687909979; E-mail: veide@kth.se

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M) was purchased from Fluka. D-glucose was purchased from Prolabo, D-xylose from Alfa Aesar, and HMF and furfural were purchased from Sigma-Aldrich. All media components were purchased from VWR or Merck Millipore.

Enzymes: Cellic® Ctec 2 (prod. no. VCS00002) and Spirizyme® Achieve (prod. no. NANY7036) were gifts from Novozymes, Denmark. Viscozyme® L (prod. no. V2010) was purchased from Sigma-Aldrich.

Microbial strain: The in-house available bacterial strain *E. coli* PPA652ara [6] was applied.

Origin and sampling of MSW

Non source-sorted MSW was collected from the Swedish waste management company Ragn-Sells AB Högbytorp, a full-scale management facility. The facility collects waste from grocery stores as well as household waste from the city of Stockholm, Sweden. The ratio is unknown. Due to large volumes of incoming waste, some of the waste is temporarily stored, and the time before processing after collection is thus unknown. The MSW is mechanically processed to produce a slurry and a solid reject. The processing steps involve breaking the packaging and initial grinding. In this step, materials (glass and plastics) are separated and rejected from the slurry. The remaining MSW slurry is normally sent to an anaerobic digestion facility. Before shipping, the MSW slurry is stored in a 140 m^3 tank. This tank is positioned outdoors and thus exposed to the seasonal climate. In this study, MSW slurry was sampled from the tank and used. The residence time in the tank before sampling was less than 24 h. The slurry was aliquoted and stored at -20°C before the experiments were performed. The MSW was collected in January and the same batch was used throughout the experiment.

Solid-liquid fractionation of MSW

Frozen MSW slurry was thawed in cold water. Then, the slurry (batch of 500 mL) was ground in a regular food processor at maximum speed for approximately 5 min at ambient temperature (20-23°C). The ground slurry was fractionated by centrifugation at 4000 x g for 15 min at 4°C (Beckman AVANTI J20 XP, rotor JA-12) to yield a solid and a liquid phase. The liquid phase (LMSW) was stored at -20°C or used directly after fractionation. The solid phase was dried at approximately 80°C overnight. To increase homogeneity, the dry solid phase of MSW, here abbreviated as DSMSW, was ground and stored at ambient temperature (20-23°C) until used. The total moisture content of the wet solid phase was analyzed.

Acid-catalyzed hydrolysis of DSMSW

To determine the solid-to-liquid ratio to be used, different amounts (g) of DSMSW were added to screw-capped glass vials with silicon packing. Sulfuric acid, 1 mL 6 % (*w/w*), was added, and the tubes were immersed in an electric heater (Grant Instruments, UK) set at 155°C. The reactions were allowed to proceed for 30 min before storing on ice.

To find the optimal parameter settings for the acid-catalyzed hydrolysis of DSMSW, design of experiments was applied. For this, the software MODDE (Umetrics, Sweden) was used for experimental set-up and evaluation. The evaluated responses were concentration of D-glucose, D-xylose, HMF, and furfural. Multiple linear regression was used to identify models. The response values were evaluated, and insignificant model coefficients were removed to maximize the goodness of prediction (Q^2). The generated models were validated according to the requirements for a good model [10].

First, temperature (121, 138, and 155°C), reaction time (10 min, 20 min, and 30 min), and the concentration of sulfuric acid (2, 4, and 6%) (*w/w*) were varied in a full factorial design. This resulted in a total of 30 experiments that included three replicates of the center points (Table SI-2). A solid-to-liquid ratio 1:10 (*w/v*) was used and a total volume of 1 mL, based on the findings in aforementioned sections. Second, the experiment was repeated but with extended factor boundaries: reaction time (30, 45, and 60 min) and sulfuric acid concentration (4%, 6%, 8%) (*w/w*) resulting in a total of 12 experiments, keeping the temperature constant at 155°C. To locate the optimal parameter settings within the design space, a built in function in MODDE called Optimizer was used (Table SI-6 for details).

Enzyme-catalyzed hydrolysis

DSMSW: For enzyme-catalyzed hydrolysis, three commercial enzyme preparations (EP); Cellic® Ctec 2 (A), Viscozyme® L (B) and Spirizyme® Achieve (C) were applied. Prior to hydrolysis, DSMSW was mixed with a sodium acetate buffer (100 mM, pH 4.5) at a solid-to-liquid ratio of 1:10 (*w/v*). Then, enzyme solutions, 250 µL EP A, 400 µL EP B, and 100 µL EP C were added in different combinations (Table SI-8), and the reaction volume was adjusted to 10 mL. The enzyme-catalyzed reactions were performed in Falcon tubes (50 mL) and incubated at 50°C at 150 rpm. The background hydrolysis reaction was measured by adding a sodium acetate buffer (100 mM, pH 4.50) instead of an enzyme solution. Also, the concentrations of soluble sugars present in the commercial enzyme preparations were measured. Both the values of the background hydrolysis reaction and the concentrations of soluble sugars present in the enzyme preparations were subtracted from the values obtained from the enzyme-catalyzed hydrolysis.

DSMSW/Enzyme load: The optimal enzyme combination (Table SI-8 no. 5) was applied in all further experiments. To ensure that an appropriate amount of enzyme was used, additional 125 µL EP A and 50 µL EP C were added after 2.5 and 5 h, respectively. The same reaction conditions as in the section above were used. To confirm that the enzymes remained active throughout the whole experiments, additional DSMSW was added in several batches. For this, 125 µL EP A and 50 µL EP C were mixed with 0.5 g DSMSW. The volume was adjusted to 5 mL with a sodium acetate buffer (100 mM, pH 4.5). After 2.5 h and 5 h additional DSMSW (0.5 g) was added.

Hydrolysis yield: To determine the hydrolysis yield at various solid-to-liquid ratios, different amounts of DSMSW were mixed together with 125 µL EP A, and 50 µL EP C in Falcon tubes (50 mL). The final volume was adjusted to 5 mL with a sodium acetate buffer (100 mM, pH 4.5). The reactions were incubated at 50 °C and 150 rpm.

Liquid phase of MSW: LMSW, 4825 µL, was mixed with 125 µL EP A and 50 µL EP C in a Falcon tube (50 mL) and incubated at 50°C and 150 rpm. The hydrolysis was performed in triplicates. A background reaction without any enzyme addition was performed using the same settings as above.

Cultivations

A frozen stock of *E. coli* PPA652ara was used to inoculate a baffled Erlenmeyer shake flask (1 L) containing 100 mL cultivation medium (minimal salt medium or LMSW). The flask was incubated at 37°C and 180 rpm. Cells that grew exponentially were used to inoculate stirred-tank bioreactors (1.5 L) containing 500 mL cultivation medium. The dissolved oxygen tension (DOT) was maintained over 20% by regulating incoming air flow and stirring rate. Sodium hydroxide (3 M) was used to maintain pH at 7. For fed-batch cultivation, the exponential feed phase (0.2 h^{-1}) was initiated as the DOT rapidly increased, which suggested that the sugar was depleted.

Cultivation media

Preparation of the LMSW medium: LMSW was sterilized at 121°C for 20 min at 1 bar overpressure. After sterilization, 1 mL L^{-1} 1 M $MgSO_4$ and 1 mL L^{-1} trace element stock solution was sterile filtered (0.2 µm, VWR Collection) and added to the medium. The trace element stock solution consisted of (per L): 0.5 g $CaCl_2$•2 H_2O, 16.7 g $FeCl_3$•6 H_2O, 0.18 $ZnSO_4$•7 H_2O, 0.16 g $CuSO_4$•5 H_2O, 0.15 g $MnSO_4$•4 H_2O, 0.18 $CoCl_2$•6 H_2O and 20.1 g Na-EDTA. Preparation of the DSMSW medium: A minimal salt medium was used for all cultivations that used DSMSW. These contained (per L): 1.6 g KH_2PO_4, 6.6 g Na_2HPO_4•2 H_2O, 7.0 g $(NH_4)2SO_4$ and 0.5 g $(NH_4)_2$-H-citrate. Sugar stock solutions were autoclaved separately before being added to the media. For shake-flask cultivations and repeated-batch cultivations an initial concentration of 5 g L^{-1} sugar was used where the ratio of D-glucose and D-xylose was 3.3:1, the same as in the hydrolyzate. As above, the cultivations were supplemented with a stock solution of 1 mL L^{-1} 1 M $MgSO_4$ and 1 mL L^{-1} trace elements. Then, hydrolyzed DSMSW or commercial D-glucose and D-xylose were added when the sugar deplete in the batch phase. In fed-batch cultivations, the reference feed consisted of 86 g L^{-1} D-glucose, and 10 g L^{-1} D-xylose. The hydrolyzate feed was obtained by hydrolyzing DSMSW (as described in Section DSMSW) in a phosphate buffer (100 mM, pH 5.8) at a solid-to-liquid ratio of 1:2. In bioreactor cultivations, 25 µL L^{-1} antifoam was added when required.

Analysis

Moisture content of solid MSW: The total moisture content of the wet solid phase of the MSW was determined using the European reference method EN 14774/15414.

Carbohydrate analysis of DSMSW: The fraction of lignin was determined by measuring the acid detergent lignin (ADL). Also, the acid detergent fiber (ADF) and the neutral detergent fiber (NDF) were analyzed. The hemicellulose content was determined by calculating NDF-ADF. The cellulose content was calculated as ADF-ADL. The analysis was performed by Eurofins Food and Feed Testing (Lidköping, Sweden).

Cell growth analysis: Samples for cell growth analysis were withdrawn and diluted with 0.9 % NaCl to reach an OD_{600} of 0.1. The samples were analyzed at 600 nm in a spectrophotometer (Thermo Scientific, GENESYS 20). The cell dry weight of fed-batch cultures was measured by adding 5 mL cell culture into a dry and weighted tube and centrifuging at 4000 x g for 15 min (HERMLE, Z 206 A). The supernatant was discarded before the cell pellet was dried at 105°C. Analyses were performed in triplicates.

Sugar and acetic acid analysis

To stop the cellular metabolism a sample of 2 mL cell suspension was withdrawn rapidly into a preweighted syringe containing 2 mL cold perchloric acid (0.13 M) [11]. The solution was centrifuged at 4000 x g for 15 min (HERMLE, Z 206 A) before 3.5 mL of the supernatant was added to 75 µL saturated potassium carbonate (500 g L^{-1}). The solution was incubated on ice for 15 min before the precipitate was removed with centrifugation at 4000 x g for 5 min. The sample was then sterile filtered using a 0.2 µm filter (VWR Collection).

High performance liquid chromatography: D-Glucose, D-xylose, acetic acid, HMF, and furfural were analyzed using High Performance Liquid Chromatography (HPLC, Waters Alliance, UK). Prior to analysis, solid residues were removed with centrifugation for 5 minutes at 16100 x g at 4°C (Heraeus Biofuge Fresco, Thermo Scientific). The remaining liquid was incubated on ice or at -20°C before analysis. Sample separation was achieved using an Aminex HPX-87 H column (Bio-Rad, USA) with a mobile phase of 0.008 N H_2SO_4 (0.5 mL min^{-1}) at ambient temperature (20-23°C). D-glucose, D-xylose, and acetic acid were detected using a refractive index detector (Waters Alliance, UK) while HMF and furfural were analyzed using a photodiode array detector (Water Alliance, UK) at 286 and 280 nm, respectively. Concentrations were calculated by the use of standards.

Results and Discussion

Solid-liquid fractionation of MSW

The solid-liquid fractionation of MSW slurry resulted in two distinct phases; a liquid-(70%, *v/v*) and a solid phase (30%, *v/v*). The wet solid phase had a moisture content of approximately 70%. Thus, the slurry had a moisture content of approximately 91%.

Carbohydrate analysis

The carbohydrate content of the DSMSW was measured to: cellulose 12%, hemicellulose 11%, starch and monomeric sugar 19 ± 15%. The lignin content was measured to 6 ± 10%.

Acid-catalyzed hydrolysis of DSMSW

Linear regression models were identified from the obtained data at the two different factor ranges tested. For details: Figures SI-2 and SI-3; Tables SI-3 and SI-5. The optimal conditions for sugar extraction were found inside the model for the first tested factor boundaries (Figure 1). The model equations (Equations SI1-SI4) suggests that increasing the sulfuric acid concentration and reaction time further could improve the extraction yield of both D-glucose and D-xylose. However, additional models contradict those implications (Figure SI-4). The optimal conditions were 155°C, 30 min, and 6% sulfuric acid. The extracted sugar concentrations were 28 and 7 g L^{-1} for D-glucose and D-xylose, respectively, at a solid-to-liquid ratio of 1:10 (*w/v*).

Enzyme-catalyzed hydrolysis of MSW

DSMSW: EP A consists of hemicellulase and cellulase activities. This enzyme preparation is known to effectively hydrolyze lignocellulosic materials [12]. EP B is a multi-enzyme complex that contains various enzymatic activities (cellulase, hemicellulase, xylanse, arabanase, and β-glucanase [13]. EP C is a glucoamylase solution developed for starch fiber matrix degradation. Consequently, it contains the catalytic activities required to degrade both linear and branched molecules. All three enzyme preparations contained a certain amount of sugars; EP A: 0.67 g L^{-1} D-glucose and 0.76 g L^{-1} D-xylose, EP B: 4.8 g L^{-1} D-glucose and 5.5 g L^{-1} D-xylose, and EP C 0.72 g L^{-1} D-glucose and 0.26 g L^{-1} D-xylose. A minor background hydrolysis (without any additions of acid or enzyme) was identified and measured (a total of 5 g L^{-1} sugar after 5 h). The minor conversion shown is probably due to elevated temperature and slightly acidic conditions (pH 4.5). Additionally, a small amount of residual sugars are present in the DSMSW as a result of the solid-liquid separation as the liquid phase contains soluble sugars. The enzymes were tested separately, in combination, and when added sequentially (Figures 2 and 3). The addition of EP A generated the highest concentration of D-xylose (6.3 g L^{-1}) but the lowest concentration of D-glucose (14 g L^{-1}), among the three enzyme preparations. The highest concentration of D-glucose (25 g L^{-1}) was generated upon the addition of EP C, while the lowest concentration of D-xylose (3.4 g L^{-1}) was generated using the same enzyme. A combination of EP A and C gave the highest concentrations of both D-glucose (28 g L^{-1}) and D-xylose (6.1 g L^{-1}). Simultaneous or sequential addition of the enzyme combinations did not affect the results significantly; neither did the choice of buffer (Table SI-10). A combination of all three enzyme preparations

did not result in an improved sugar yield. The enzymes remained active throughout the reaction, and the yield was not increased for D-glucose when additional enzymes were added (Table SI-9). In contrast, the concentration of D-xylose increased. The obtained yields of sugar using enzyme-catalyzed hydrolysis correspond well to the amounts obtained by applying acid hydrolysis. Thus, the enzyme-catalyzed hydrolysis is as effective as acid-catalyzed hydrolysis.

Liquid phase of MSW: Non-hydrolyzed LMSW contained a total amount of 22 g L^{-1} sugars available for *E. coli* growth (Table 1). Thus, there were a certain amount of soluble sugars present in the LMSW. By applying hydrolytic enzymes (EP A, EP C), the concentration of fermentable sugars increased to 36 g L^{-1} after 2.5 h of incubation. The concentration of D-glucose increased 2-fold (from 10 g L^{-1} to 21 g L^{-1}) after only 2.5 h of incubation while the amount of D-xylose increased to a minor extent (13 g L^{-1} to 15 g L^{-1} after 5 h). These results indicate that the LMSW can be exploited as a source of carbon and energy in both batch and continuous-mode cultivation.

Increasing sugar concentration

The DSMSW load (1:100-1:2 *w/v*, Figure 4) affected the yield of released D-glucose and D-xylose per gram of DSMSW (from the enzyme-catalyzed reaction). The yield was negatively affected by an increase in the solid-to-liquid load. However, between a solid-to-liquid ratio of 1:10 and 1:2, only a minor drop in yield was observed for both D-glucose and D-xylose. Similar yields of sugar were obtained for the acid-catalyzed hydrolysis. For the enzyme-catalyzed hydrolysis, the yield could not be improved by increasing the enzyme concentration (Table SI-9). This suggests that the hydrolysis had reached its limits.

Interestingly, below a ratio of 1:10 the yield increased dramatically with a decreased in solid-to-liquid ratio, which could be due to the formation of inhibitory by-products at high ratios. However, due to the abundant and cheap nature of MSW, obtaining a high yield is not a priority. The potential of using the solid residue in other applications could be explored to increase the overall process profitability.

The highest DSMSW load that could be used (solid-to-liquid ratio 1:3 *w/v*) for the acid-catalyzed reaction generated 69 g L^{-1} D-glucose and 17 g L^{-1} D-xylose. For the enzyme-catalyzed hydrolysis (solid-to-liquid ratio 1:2 *w/v*), 100 g L^{-1} D-glucose and 14 g L^{-1} D-xylose were obtained. The high solid-to-liquid ratio resulted in thick process liquids, which can entail difficulties in mixing. The sugar yields in the enzyme-catalyzed reaction were more sensitive to the solid-to-liquid ratio than the acid-catalyzed hydrolysis. Thus, the potential to release sugars might be underestimated in the acid-catalyzed reaction for solid-to-liquid ratios below 1:10 (*w/v*).

MSW as source of carbon and energy in *E. coli* cultivations

LMSW

First, LMSW (unhydrolyzed) was used as a cultivation medium without any supplementations in batch mode. This resulted in a lag phase of 4 h followed by a low growth rate (data not shown). Then, LMSW (unhydrolyzed) was supplemented with trace elements and magnesium before application as a cultivation medium in batch mode (Figure 5). This resulted in an initial exponential cell growth phase with a growth rate of 0.38 h^{-1}.

This growth rate is similar to previously published data in which

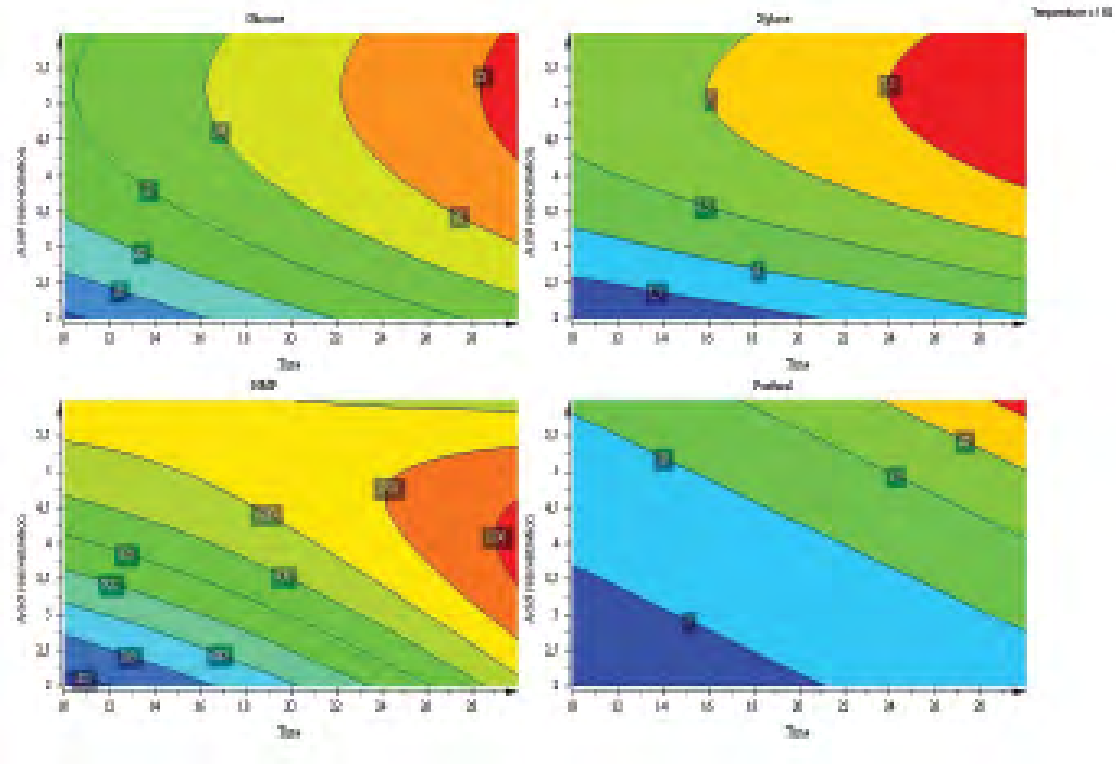

Figure 1: Representation of modeled response values (g L^{-1}) as contour plots for acid-catalyzed hydrolysis with the parameters temperature (121, 138, and 155°C), concentration of sulfuric acid (2, 4, and 6% *w/w*), and time (10, 20, and 30 min) varied in a full factorial design. Contour plots with temperature at 155°C are displayed.

Table 1: Enzyme-catalyzed hydrolysis[a] of LMSW using Cellic® Ctec 2 (A) and Spirizyme® Achieve (C) and the effect on D-glucose and D-xylose concentration during time course together with non-hydrolyzed LMSW. The reactions were performed in triplicates. The numbers are presented with the sugars from the enzyme solutions subtracted.

	D-glucose (g L^{-1})			D-xylose (g L^{-1})		
Time	**0 h**	**2.5 h**	**5 h**	**0 h**	**2.5 h**	**5 h**
Liquid phase	-	9.9	10	12	13	13
A+C	-	-	21	-	15	15

[a] **Reaction conditions** 4825 µL LMSW, 125 µL EP A, 50 µL EP C, 50ºC and 150 rpm

D-glucose is used as the sole carbon source for the parental strain PPA652 [7]. The reduced growth rate (0.8 to 0.38 h^{-1}) is a result of the deleted high affinity glucose uptake system ptsG. A higher growth rate (0.59 h^{-1}) was obtained using a mixture of D-glucose, D-xylose and L-arabinose [6]. In the present study, the growth in minimal medium supplemented with D-glucose and D-xylose was measured to 0.56 h^{-1} (Figure 6). Thus, the presence of LMSW lowered the cellular growth rate slightly.

Although the elementary analysis of LMSW shows a natural occurrence of trace elements (data not shown) it does not contain enough (or the right proportions) of soluble trace elements to support the growth of *E. coli*. It could not be determined if the slight decrease in cellular growth rate is due to the nature of LMSW or if some of the supplemented metal ions caused inhibitory effects.

The uptake of D-xylose should increase the cellular growth rate, but in the presence of LMSW, the D-xylose consumption rate was lower than data previously published [6]. The initial growth indicates that the LMSW medium does not seem to contain any toxic components that hinder growth. After 6 h of exponential cell growth the growth ceased and entered a second growth phase in which the growth rate was low. This could be due to nutrient deficiency or the formation of an inhibitory substance. However, neither D-glucose nor D-xylose was a limiting factor during the cultivation.

This shows that *E. coli*, to some extent, can utilize both D-glucose and D-xylose as a carbon source in the presence of the other components present in LMSW. However, further investigation of inhibitory or restrictive substances is necessary. This is required in order to fully utilize all sugars present in LMSW and thus achieve higher productivity.

The LMSW had an inherent concentration of acetic acid of 1 g L^{-1}. Acetic acid can be produced by the decomposition of hemicellulose. Also, acetic acid can originate from microorganisms present in the MSW before sterilization. The initial concentration of acetic acid was not an inhibiting factor, however, it could be a drawback because acetic acid is also produced during cellular growth.

The D-xylose consumption rate was constant over the growth rate and was coupled to cell growth. For D-glucose, the rate increased to the double between 6-12 h, compared to 2-6 h of cultivation. The same pattern was seen for the production of acetic acid.

DSMSW

Increasing the solid-to-liquid ratio (1:2 *w/v*) during the enzyme-catalyzed hydrolysis of DSMSW, concentrations up to 114 g L^{-1} of fermentable sugars were achieved. The high sugar content shows on potential application of DSMSW in fed-batch cultivations, in which a concentrated substrate feed is required. This shows on a potential application of fractionated MSW in fed-batch cultivations which requires a concentrated substrate feed. When selecting lower solid-to-liquid ratios lower concentrations of sugar are generated that can beneficially be used as a carbon source for batch or continuous-mode cultivations. By fine-tuning the preparation procedure of DSMSW, a more defined chemical background compare to the one of LMSW, can be obtained. To investigate the potential of using hydrolyzed DSMSW as a source of carbon and energy in *E. coli* cultivations, repeated batch experiments were conducted. Both cultivations displayed the same behavior in the minimal media batch phase (0-6 h) and the sugar depleted simultaneously (Figure 6), also indicated by an increase in the DOT-signal (data not shown).

In the reference culture, both D-glucose and D-xylose were consumed simultaneously (Figure 6A). No prominent lag phase was detected after the addition of batch sugar. When hydrolyzed DSMSW was added, D-glucose was consumed, while D-xylose was not taken up and thus accumulated (Figure 6B). This suggests that the hydrolyzed DSMSW contained a metabolite that inhibited the uptake of D-xylose. Also, a short lag phase in growth was seen on the OD_{600} curve. This was further supported by the DOT- and pH-signal (data not shown), as no titration was required. According to the DOT-signal, the first lag phase was 1.5 h and the second was 0.9 h. However, more cells were present at the second addition, and the effect of addition was not as

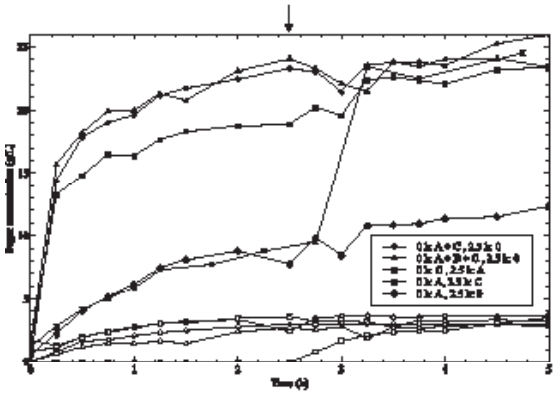

Figure 2: Enzyme-catalyzed hydrolysis of DSMSW with addition of Cellic® Ctec 2 (A) and Spirizyme® Achieve (C), at the reaction start (0 h) or after 2.5 h and the produced concentrations of D-glucose (filled legends) and D-xylose (open legends) during time course. The second enzyme addition is indicated by the arrow. The symbols are connected for clarity.

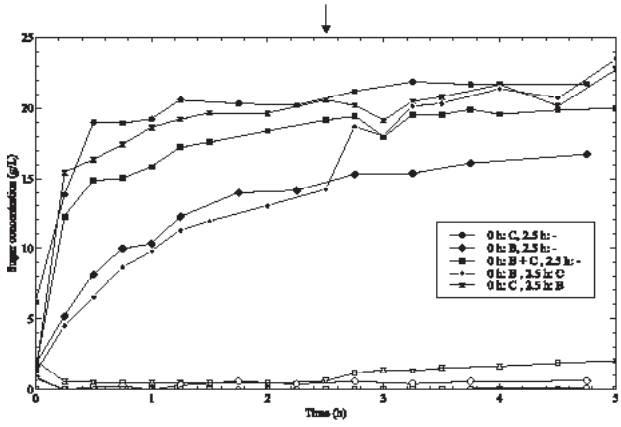

Figure 3: Enzyme-catalyzed hydrolysis of DSMSW with addition of Viscozyme® L (B) and Spirizyme® Achieve (C), at the reaction start (0 h) or after 2.5 h and the produced amount of D-glucose (filled legends) and D-xylose (open legends) during time course. The second enzyme addition is indicated by the arrow. The symbols are connected for clarity.

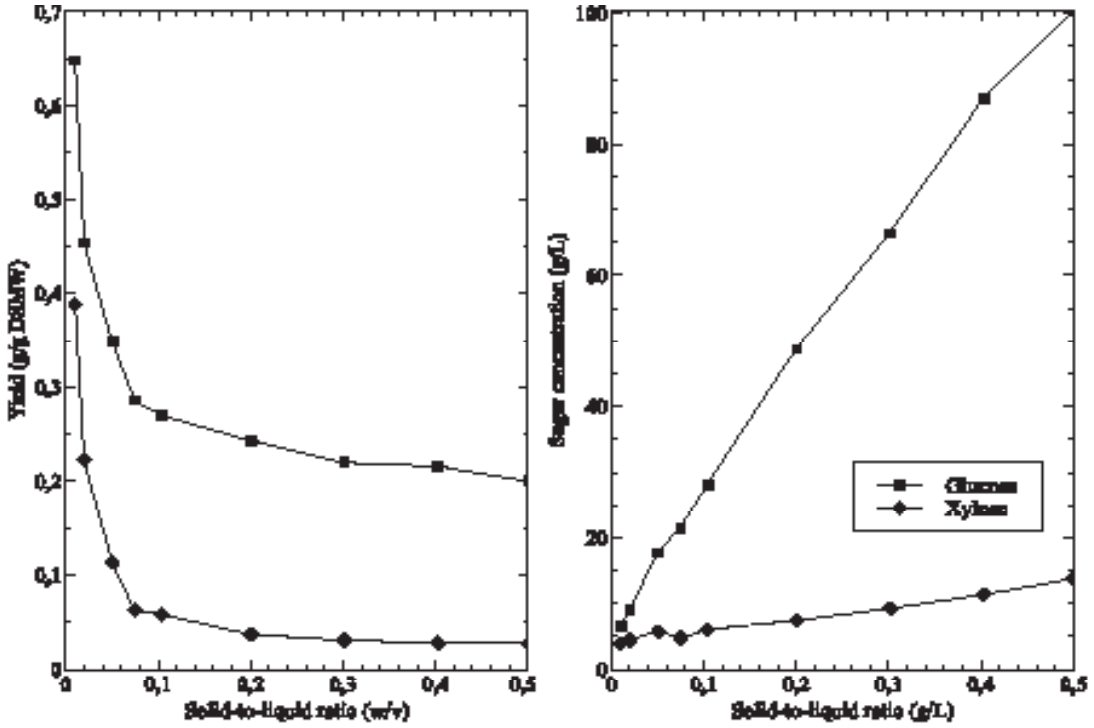

Figure 4: Yield (g sugar g DSMSW-1) and concentration of sugars (g L^{-1}) generated by enzyme-catalyzed carbohydrate hydrolysis after 5 h of incubation using Cellic® Ctec 2 and Spirizyme® Achieve.

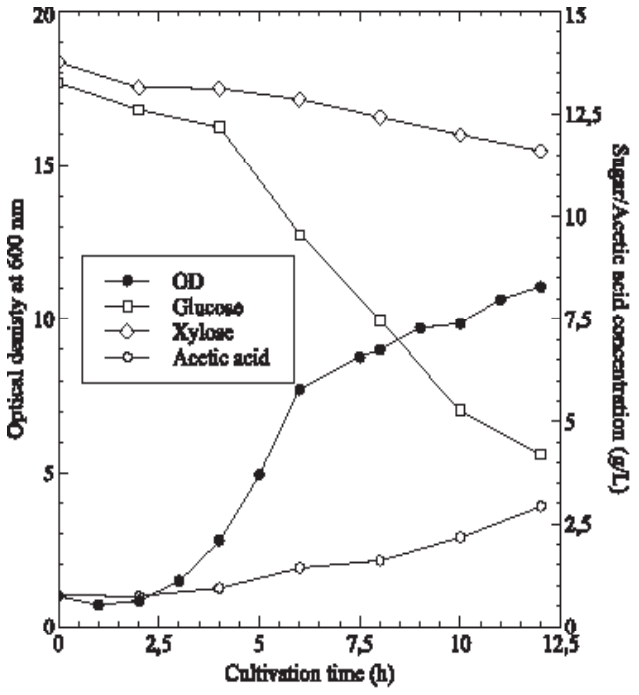

Figure 5: Growth of *E. coli* PPA652ara on LMSW supplemented with trace elements and magnesium in batch mode. Growth rate of 0.5 h^{-1} in exponential phase (1-6 h), R^2=0.984.

prominent. Thus, the lag phase was determined to be the time over OD_{600}. The lag phase was calculated to be 0.25 h OD_{600} h^{-1} and 0.007 h OD_{600} h^{-1}, for the first and second additions of hydrolyzed DSMSW, respectively. Thus, the lag phase was reduced at the second addition. The shorter second phase indicated that the cells adjusted to the new environment. However, D-xylose was, nevertheless, not consumed. To test the aforementioned theory that D-xylose uptake is inhibited, a fed-batch cultivation using a low feed rate of 0.2 h^{-1} was conducted to dilute the effect of the plausible inhibitory metabolite. No prominent difference in cell growth pattern was detected between the reference using commercial sugars and the cultivation using hydrolyzed DSMSW as the feed (Figure 7). The pentose sugar, D-xylose, was effectively consumed in both the reference cultivation and in the one using hydrolyzate DSMSW as the feed (Figure 8).

Conclusions

Future implementation for MSW as a source of carbon and energy in biorefinery platforms depend on the possibility of making the carbohydrate content accessible for cellular uptake. This study explored the potential of Swedish MSW collected from a full-scale management facility as a renewable carbohydrate source for source for sugars, in both batch and fed-batch *E. coli* cultivations. To alter the sugar concentration in MSW hydrolyzate, different fractions of MSW were explored.

It was demonstrated that MSW slurry can be manipulated in order to generate sugar concentrations from 20 g L-1 up to 114 g L-1 by applying fractionation and hydrolysis. For an application in which lower concentrations are desirable, the liquid phase of MSW might be used directly. However, further investigation of inhibitory or limiting substances is required. The MSW fractionation seemed to serve two purposes: 1) To remove liquid and thus enable a hydrolyzate of high sugar concentration, and 2) To change the chemical background of the obtained medium. To obtain higher sugar concentrations, solid-to-liquid fractionation can be used combined with drying.

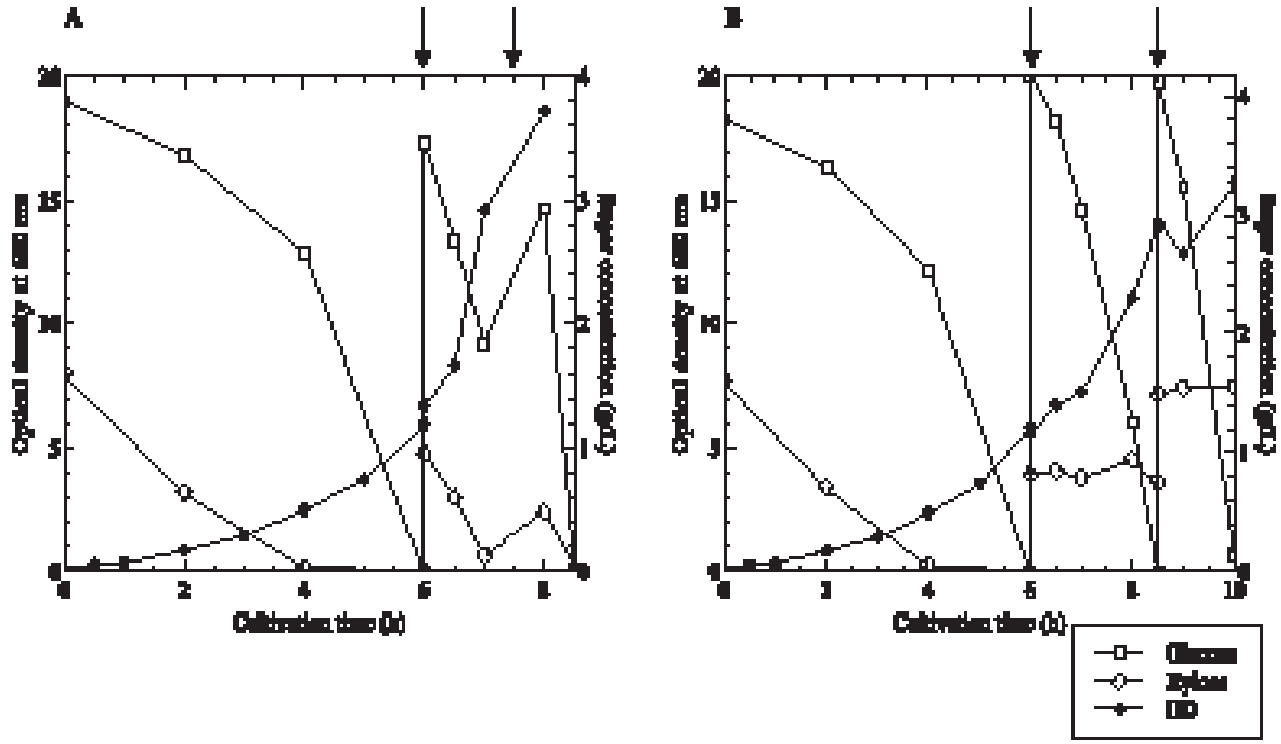

Figure 6: Repeated batch cultivations of *E. coli* PPA652 with commercial D-glucose and D-xylose (A) and hydrolyzed DSMSW (B). Addition of sugar solutions indicated by arrows. Growth rate in first batch phase equal in both A and B of 0.56 h^{-1} (R^2=0.984 and 0.984, respectively. Growth rate after addition of sugar calculated to 0.52 h^{-1} (A, R^2=0.998) and 0.27 h^{-1}, R^2=0.936).

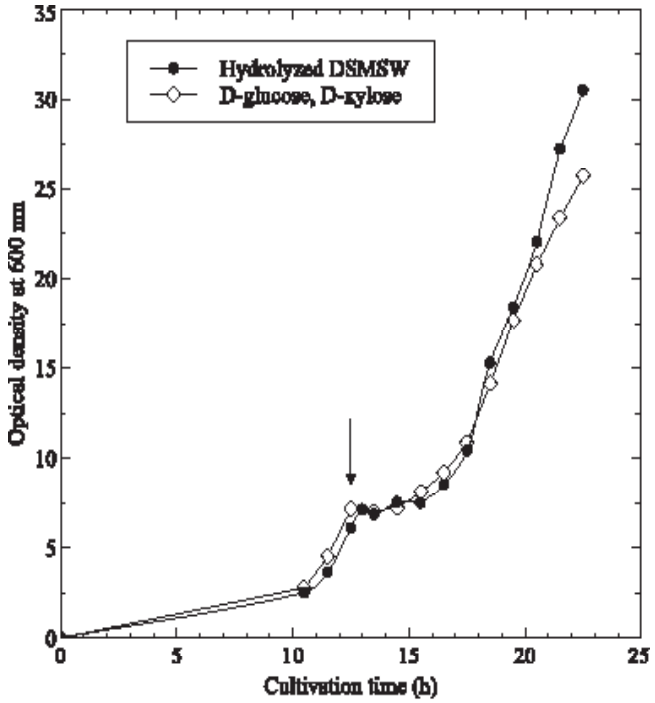

Figure 7: Fed-batch cultivation of *E. coli* PPA652ara using commercial D-glucose and D-xylose or hydrolyzed DSMSW as feed. Initial batch phase using minimal medium supplemented with a total of 5 g L^{-1} commercial D-glucose and D-xylose (ratio 3.3:1). Growth rate in batch phase: 0.47 h^{-1} (R^2=0.999).

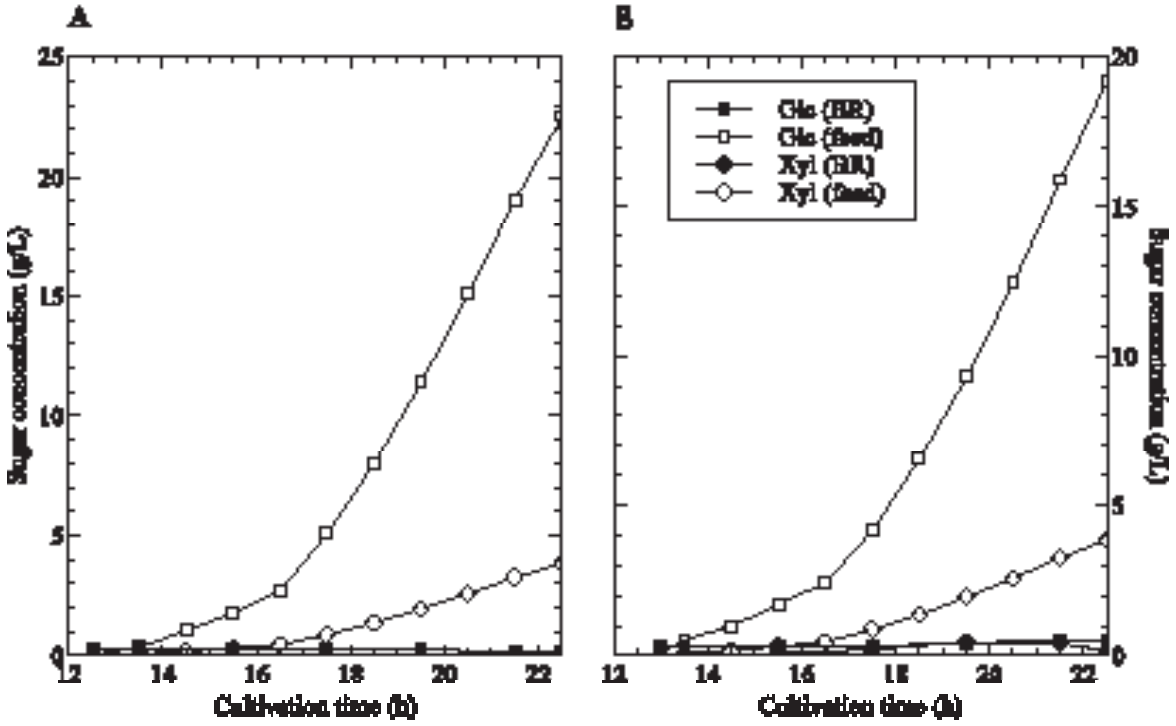

Figure 8: Fed-batch cultivation of *E. coli* PPA652ara using commercial D-glucose and D-xylose or hydrolyzed DSMSW as feed. Concentration of sugar added in feed and measured in bioreactor (BR).

Thereby, water will be removed and the carbohydrate content will be concentrated. Finally, it was demonstrated that the majority of the sugar present in MSW was consumed by the cells using a fed-batch cultivation technique. The optimal growth rate in fed-batch cultivation mode, with maintained dual sugar assimilation, remains to be explored.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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