Lignocellulose Feedstocks for the Production of Lactic Acid

M. Neureiter, H. Danner*, L. Madzingaidzo, H. Miyafuji, C. Thomasser, J. Bvochora, S. Bamusi, and R. Braun
Department of Environmental Biotechnology, Institute of Agrobiotechnology, Konrad Lorenz Str. 20, A-3430 Tulln, Austria

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Conventional substrates based on starch or sucrose are not available in sufficient quantities and at reasonable prices to provide the feedstock for the production of chemical bulk products. Lignocellulosic materials are an attractive alternative since they are abundant and usually low-priced. However, it is comparatively difficult to convert these materials into fermentable sugars. The conversion process usually includes high temperatures and/or the use of chemicals, which leads to the liberation of compounds that are toxic to microorganisms. In order to use the substrate most efficiently, it appears to be necessary that also hemicellulose derived pentoses are utilized in the fermentation process.

This paper presents a process for the production of lactic acid from lignocellulosic biomass, which is based on the research conducted at IFA-Tulln in the past years. The use of lignocellulose as fermentation feedstock requires: 1) an effective treatment in order to obtain fermentable sugars, 2) a detoxification procedure, which results in a fermentable sugar solution, and 3) a fermentation process providing high yields and productivities from the respective substrate. The presented data include the evaluation of softwood and straw regarding their suitability for dilute acid pretreatment and the determination of optimal hydrolysis conditions for the recovery of hemicellulose derived sugars. Furthermore, toxic compounds from different hydrolysates have been identified and methods for the detoxification of hydrolysates are discussed. It could be demonstrated that dilute acid hydrolysates from lignocellulosic raw materials can be fermented to lactic acid by thermophilic *Bacillus* strains. An overall process including continuous fermentation in a membrane bioreactor including product purification by electrodialysis is introduced and evaluated.

Keywords
Lignocellulose, dilute-acid hydrolysis, lactic acid, fermentation, thermophilic strains

Introduction

The replacement of fossil materials for the production of energy and chemicals is a challenge that we will have to face in the near future. Although crude oil resources will still cover the demand for at least the next 40 years, it is a fact that these resources are limited, and it is expected that the crude oil prices will increase substantially within the next decade.[1] Therefore, renewable resources for the production of chemicals have to be made accessible. The only sustainable resource is biomass, since it can be regrown after harvest.

Apart from thermochemical conversion like pyrolysis or gasification, biotechnological processes, which are mainly based on sugars, have a great potential for the production of chemicals from biomass. *Danner* and *Braun*[2] stated that a large variety of chemicals that are currently based on fossil resources can be produced from biomass via fermentation or by chemical synthesis departing from fermented products. In this context the focus is on so called key intermediates, which can be produced by fermentation at low cost and serve as feedstock for further syntheses. Lactic acid has the best prerequisites to become an intermediate product, since its functional groups make it susceptible to further reactions, e.g. the production of propionic acid, acetic acid, acrylic acid, alanin, or pyruvic acid.[3]

The world market for lactic acid is estimated at 54 500–59 000 t per year,[4] whereas the traditional applications are as acidulant and the production of emulsifiers in the food industry. The demand is expected to increase due to the growing production of lactic acid polymers (PLA). These polymers, which are produced from lactic acid or its dimer dilactide, respectively, have the potential to replace part of conventional plastics. The applications of lactic acid polymers range from packaging material to

*Author to whom all correspondence should be addressed
E-Mail: danner@ifa-tulln.ac.at
Tel: +43 2272 66280 558, Fax: +43 66280 503

textile fibers and biomedical applications, whereas these products have the advantage that they are biodegradable. Cargill Dow Polymers LLC recently erected a production plant for lactic acid polymers in Blair/Nebraska (USA), which has a capacity of 140 000 t PLA per year,[5] and therefore respective amounts of lactic acid will have to be supplied as feedstock.

The production of PLA requires lactic acid of high chemical and optical purity. The physical and biological properties of lactic acid polymers are related to the enantiomeric purity of lactic acid stereocopolymers.[6] While homopolymers form regular structures and develop a crystalline phase, copolymerization with D- or L-lactides or lactic acid leads to interruption of the regular structures and the formation of amorphous materials. In order to achieve the desired polymer properties, high purity D- or L-lactic acid monomers are necessary.

The most important raw materials for lactic acid fermentation are currently sucrose (molasses) or glucose from starch hydrolysates. However, the amounts of these materials are not high enough to replace fossil resources by biomass on the long term. Furthermore, sucrose and starch are used in food processing and therefore prices compete with those of the food industry. A possible alternative are lignocellulosic feedstocks, which are available at large quantities and comparably lower cost, and have thus the potential to replace fossil resources.[7] A wide use of lignocellulose as fermentation feedstock is currently inhibited by the high costs for the conversion of its polysaccharides into fermentable sugars. Since cellulose and hemicellulose are more recalcitrant to enzymatic hydrolysis than e.g. starch, a physical and/or chemical treatment step is necessary in order to obtain a material that can be subjected to enzymatic hydrolysis. For the enzymatic conversion high amounts of enzymes and high enzyme prices have to be taken into consideration.[8] However, enzyme prices are expected to be reduced by the factor ten within the next five years due to support of a $ 32 million grant by US-DOE to the enzyme producers Novozymes and Genencor.[9]

During the past years intensive research in the fields of lactic acid fermentation and purification and in the utilization of lignocellulosic biomass as fermentation feedstock has been conducted at IFA-Tulln. The subject of this publication is the optimization of dilute acid hydrolysis of softwood chips and the fermentability of hydrolysates which are rich in hemicellulose derived sugars by a thermophilic *Bacillus* strain.

Materials and methods

Determination of sugars and organic acids

Sugar analysis was performed with high performance liquid chromatography (HPLC) with refractive index detection, using a Thermo Hypersil HyperREZ XP Carbohydrate Pb column at a flow rate of 0.6 ml min^{-1}, column temperature of 85 °C, and water as solvent. This system was used for the analysis of cellobiose, glucose, mannose, xylose, arabinose, and the sugar degradation products furfural and 5-hydroxymethylfurfural.

Organic acids and alcohols were also determined by HPLC using a Merck Polysphere OA KC RT 300–700 column at a flow rate of 0.4 ml min^{-1}, column temperature of 42 °C and 0.01 mol l^{-1} H_2SO_4 as solvent. Fermentation samples were also analyzed with this system.

Determination of carbohydrate polymers and lignin

The carbohydrate and Klason lignin content of lignocellulosic materials were determined after a complete acid hydrolysis which was performed according to a modified TAPPI standard method,[10] where 0.3 g of the material were treated with 3 ml of w = 72 % sulfuric acid at 30 °C for 60 min, followed by a dilution with 87 ml water and pressure cooking at 120 °C for 20 min. Extractives were not determined. Sugars were analyzed by HPLC after a neutralization of the solution with $Ba(OH)_2$. Klason lignin, which is the material that remains insoluble after hydrolysis with sulfuric acid, was separated by filtration and determined after drying at 105 °C for 1 day.

In order to account for dissolved oligomers in liquid hydrolysates a post hydrolysis step was performed. The solutions were brought to an acid fraction of 2.4 % by the addition of concentrated sulfuric acid. The reaction was carried out in standard laboratory glass tubes with 4–6 ml of hydrolysate each. The tubes with the hydrolysates were autoclaved at 120 °C for 20 min. After neutralization with $Ba(OH)_2$ the sugars could be determined by HPLC.

Composition of lignocellulosic material

Raw materials for the production of hydrolysates were spruce chips (*Picea abies*) with a particle size of 1 mm, and straw (*Triticale*) from a regional farm, which was chopped to an average length of 1 cm. Both materials were stored in a dry place and had a dry matter content of w = 90 %. The composition of straw and softwood is given in Table 1.

Table 1 – *Composition of lignocellulosic raw materials in g 100g^{-1} dry matter*

	Softwood (*Picea abies*)	Straw (*Triticale*)
Klason Lignin	28.38	21.11
Glucan	38.57	37.05
Xylan	4.99	17.34
Galactan	1.93	1.35
Arabinan	0.96	2.51
Mannan	9.29	0.95

Determination of fermentation inhibitors

Phenolic compounds in the hydrolysates were extracted with ethyl acetate for 30 min under ultrasonification. The extractions were repeated four times with fresh ethyl acetate. The extracts were dried with Na_2SO_4 and mixed with N,O-bistrimethylsilyltrifluoroacetamid containing w = 1 % of trimethylchlorosilane. This mixture was treated at 50 °C for 1 h to convert the phenolic compounds into volatile trimethylsilyl derivatives, which were analyzed by gas chromatography and mass spectrometry (GC-MS) as described by *Miyafuji* et al.[11]

For a quick estimation on the presence of phenolic compounds in the hydrolysates the absorbances at 280 nm were measured with a spectrophotometer.[12]

Statistical experiment design

The dilute acid hydrolysis of softwood chips was optimized using a face centered 3^2 central composite design with starpoints. The parameters of investigation were sulfuric acid concentration (0.02 and 0.06 mol l^{-1}), reaction temperature (160 and 180 °C), and reaction time (8 and 20 minutes). The experimental error was determined at the centerpoint (0.04 mol l^{-1} acid, 170 °C, 14 min), which was repeated three times. Experiments were carried out in fully randomized run order. The data were evaluated with statistical software (Statgraphics 5.0). The significance of effects and two factor interactions was estimated by ANOVA. The data were used to calculate a second order response surfaces as shown in Figs. 1 and 2.

Dilute acid hydrolysis

Hydrolysis experiments were carried out in a 20 l batch hydrolysis reactor which has been described before.[13] The effect of acid concentration, temperature, and reaction time on the yield of monosaccharides, sugar degradation products and organic acids from softwood chips was investigated according to the statistical experiment design. The concentration of dry solids in the reaction mixture was kept constant at 15 %. For these experiments the reactor was filled with 0.50 kg of wood dry matter and mixed with 2.833 l of acid in the required concentration. The average time to reach the requested temperature was 10 min. The experiments were terminated by opening a ball valve to allow the reactor contents to expand into a 100 l container which was filled with 12 l of water to cool down the reaction mixture to a temperature below 70 °C and to avoid losses due to evaporation. The remainings in the reactor were washed out with 5 l of water and this solution was mixed with the rest of the broth. The liquid phase was separated by pressing through a filter cloth leaving a solid press cake with approximately w = 25 % dry matter substance.

For the fermentation tests hydrolysates from straw and softwood were produced at 170 °C and a reaction time of 12 min. 1.1 kg of the material (90 % dry matter) were mixed with 5.14 l of sulfuric acid (c = 0.05 mol l^{-1}), which results in a dry matter

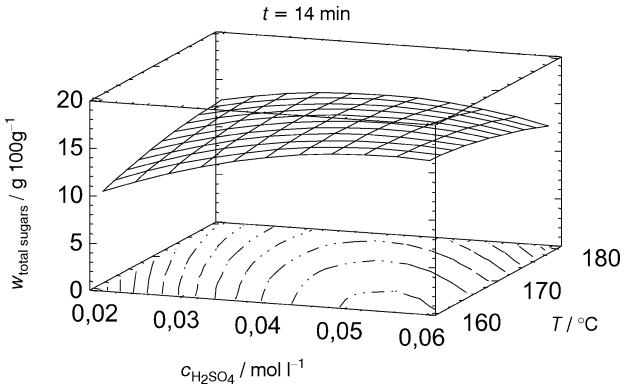

Fig. 1 – *Estimated response surface for total sugar yield showing the influence of sulfuric acid concentration and temperature at a reaction time of 14 min. Dotted lines are lines of equal yield. R^2 for the calculated model is 85.6 % .*

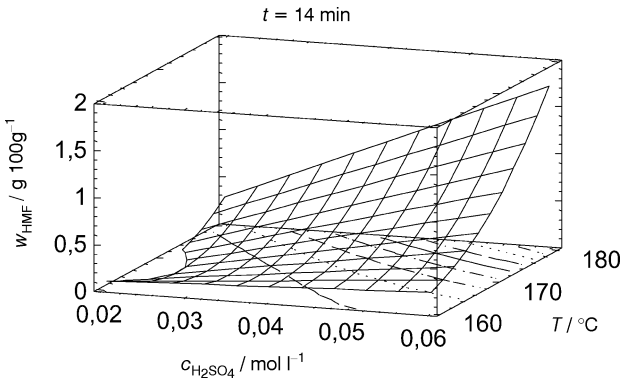

Fig. 2 – *Estimated response surface for 5-hydroxymethylfurfural showing the influence of sulfuric acid concentration and temperature at a reaction time of 14 min. R^2 for the calculated model is 95.7 %.*

fraction of w = 16 %. In this case the expansion tank was not filled with water and the reactor was not washed out, which leads to losses due to evaporation and material that is left in the reactor. By this way hydrolysates with higher sugar concentrations can be obtained. However, the losses during evaporation would lead to lower yields compared to an optimized process including the condensation of the evaporate. As a consequence no yields were calculated for these experiments but it can be assumed that the yield values determined in the statistical experiment design are applicable to these experiments.

Detoxification

The hydrolysates obtained from straw and softwood were subjected to a treatment called overliming, which is used very often for the detoxification of dilute acid hydrolysates.[14,15] The pH of the hydrolysate was adjusted to 10 by addition of $Ca(OH)_2$ in solid form. Then concentrated sulfuric acid was added until the solution reached pH 6. The precipitate formed was removed by centrifugation and the supernatant was used for the fermentation experiments.

To a part of the samples additionally 50 g of activated carbon (Loba) were added per Litre of hydrolysate and the mixture was shaken for 30 min at ambient temperature. The solids were removed by centrifugation and the supernatant was used for the fermentation experiments.

Microorganism

Lactic acid fermentations were performed with the *Bacillus* strain BS121, which is one of several thermophilic xylose fermenting strains that were isolated from compost and silage.[16] The strain was conserved in nutrient broth (Merck) with φ = 15 % glycerol at –80 °C.

Cultivation temperature was 60 °C. Inocula were prepared on 9 g l^{-1} nutrient broth (Merck) supplied with 4 g l^{-1} yeast extract (Sigma) and 3 g l^{-1} xylose and were incubated overnight without shaking. The optical density at 700 nm (OD_{700}) of the inoculum was adjusted to 0.2 with sterile distilled water to ensure the comparability of the fermentation experiments.

Fermentation

The fermentations were performed under anaerobic conditions in stirred 100 ml glass reactors using a multi fermentation system. pH was measured continuously with electrodes and regulated to 6.0 by automatic addition of 2 mol l^{-1} NaOH. The incubation temperature of 60 °C was controlled by placing the reactors in a regulated water bath. Fermentation experiments were carried out as duplicates. The detoxified hydrolysates were diluted so that the overall sugar mass concentration amounted to approximately 30 g l^{-1} and 15 g l^{-1}, respectively. For the experiments with 15 g l^{-1} sugars, both, overlimed and activated carbon treated solutions were used, while for the 30 g l^{-1} only activated carbon treated hydrolysates were employed. The diluted hydrolysates were autoclaved and 40 ml were mixed with 8 ml sterile nutrient solution containing 54 g l^{-1} nutrient broth (Merck) and 24 g yeast extract (Loba), so that the final concentration was 9 g l^{-1} nutrient broth and 4 g l^{-1} yeast extract. 5 ml of inoculum were added and samples were taken after 0, 24, 48 and 72 h. The experiments with softwood hydrolysate were stopped after 48 h.

Results

Statistical experiment design with softwood

The results of the hydrolysis experiments are summarized in Table 2. The highest sugar yields were obtained in the centerpoints with an average of 16.42 g monosaccharides per 100 g of dry softwood. The effects of the investigated parameters on sugar mass concentration and the formation of 5-hydroxymethylfurfural (HMF) are visualized in the standardized Pareto charts. The acid concentration is the only quantity that shows a positive significant effect on the sugar mass concentration (Fig. 3). There is a strong negative interaction between temperature and reaction time and also a negative interaction between temperature and acid concentration. For the formation of HMF, which is the most important sugar degradation product in softwood hydrolysates, almost all effects and interac-

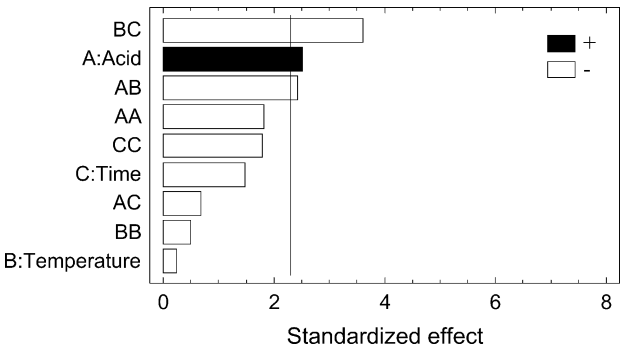

Fig. 3 – *Standardized Pareto chart for total sugar yields Standardized effects are calculated by dividing the effect by its standard error. Positive effects are symbolized by full bars, while negative effects are represented by empty bars. Effects below the line at 2.31 are not significant at the 95 % confidence level. AA, BB, CC are quadratic effects. If these are significant the respective effect is non-linear.*

Table 2 – *Yields of monosaccharides and sugar degradation products (g 100g^{-1} dry softwood) after dilute acid hydrolysis in dependence on the hydrolysis parameters acid concentration, reaction temperature (T), and reaction time (t), n.d.: not detectable*

Sulfuric acid c / mol l^{-1}	T °C	t min	Total sugars w / g 100g^{-1}	Mannose w / g 100g^{-1}	Xylose w / g 100g^{-1}	Glucose w / g 100g^{-1}	Arabinose w / g 100g^{-1}	Galactose w / g 100g^{-1}	HMF w / g 100g^{-1}	Furfural w / g 100g^{-1}	Soluble oligosaccharides w / g 100g^{-1}
0.02	160	8	6.04	1.34	2.54	0.60	0.81	0.75	0.01	0.01	7.78
0.02	160	20	10.76	3.67	3.86	1.19	0.80	1.24	0.03	0.10	6.56
0.02	180	8	14.12	5.05	4.65	2.14	0.87	1.41	0.12	0.16	4.05
0.02	180	20	7.51	3.14	2.01	1.25	0.44	0.65	0.28	0.21	9.24
0.06	160	8	14.71	6.56	3.83	2.12	0.83	1.38	0.09	0.13	n.d.
0.06	160	20	15.32	6.69	3.89	2.47	0.91	1.36	0.37	0.48	n.d.
0.06	180	8	14.37	5.63	3.30	3.52	0.78	1.14	1.04	1.01	n.d.
0.06	180	20	8.56	2.62	1.22	3.50	0.50	0.73	2.37	2.04	n.d.
0.04	170	8	13.20	5.77	3.52	1.97	0.74	1.20	0.10	0.10	5.25
0.04	170	20	12.20	5.25	3.18	2.16	0.61	0.99	0.33	0.39	4.29
0.04	160	14	13.56	6.00	3.60	1.89	0.78	1.28	0.05	0.14	2.39
0.04	180	14	14.56	5.85	3.48	3.18	0.88	1.17	1.03	1.01	n.d.
0.02	170	14	13.03	4.89	4.18	1.71	0.83	1.43	0.06	0.17	n.d.
0.06	170	14	12.31	5.14	3.09	2.40	0.67	1.01	0.50	0.56	n.d.
0.04	170	14	16.31	7.15	4.26	2.60	0.95	1.35	0.36	0.20	n.d.
0.04	170	14	15.09	6.61	3.91	2.44	0.87	1.26	0.38	0.28	n.d.
0.04	170	14	17.79	7.77	4.57	2.86	1.09	1.51	0.40	0.37	n.d.
0.04	170	14	16.48	7.13	4.23	2.73	0.98	1.42	0.44	0.82	n.d.

tions are positive and significant (Fig. 4). Temperature shows the strongest effect followed by acid concentration. There is also a strong positive interaction between these two parameters. The consequences of these effects are demonstrated in the response surface graphs, where the maximal sugar yield is predicted at low temperatures and high acid concentrations (Fig. 1). Fig. 2 shows that HMF formation increases rapidly when temperatures are elevated.

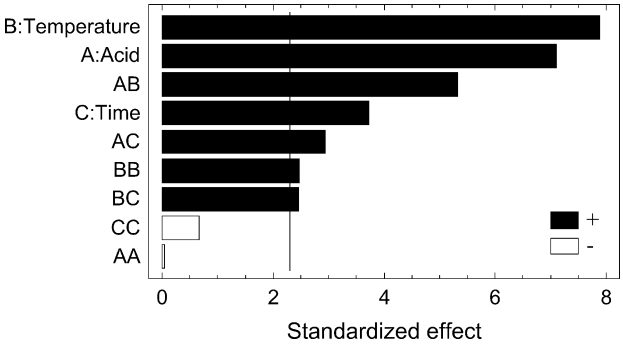

Fig. 4 – *Standardized Pareto chart for 5-hydroxymethylfurfural*

Detoxification of wood and straw hydrolysates

Table 3 shows the composition of the wood and straw hydrolysate before detoxification. The main sugar degradation product in straw hydrolysate is furfural that is generated from arabinose and xylose that make up the largest part of straw hemicellulose, while softwood hydrolysate shows high concentrations of HMF, which is derived from mannose.

Figs. 5 and 6 depict the reduction of the sugar degradation products and of phenolic compounds measured by OD at 280 nm. It has to be taken into account that the hydrolysates were diluted to a sugar mass concentration of 30 g l^{-1} and 15 g l^{-1}, respec-

Table 3 – *Composition of untreated dilute acid hydrolysates*

	Glucose γ / g l^{-1}	Xylose + Mannose γ / g l^{-1}	Arabinose γ / g l^{-1}	Acetic Acid γ / g l^{-1}	HMF γ / g l^{-1}	Furfural γ / g l^{-1}
Straw	5.398	36.185	4.321	2.969	0.136	0.913
Softwood	7.478	31.956	1.976	3.721	0.757	0.592

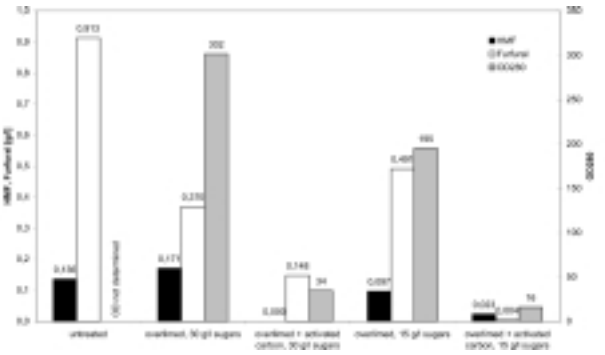

Fig. 5 – *OD at 280 nm as indicator for the content of fermentation inhibitors and concentrations of furfural and hydroxymethylfurfural in untreated straw hydrolysate and in the diluted solutions after overliming and activated carbon treatment.*

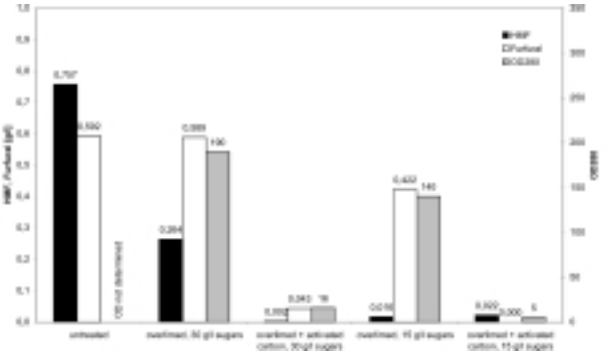

Fig. 6 – *OD at 280 nm as indicator for the content of fermentation inhibitors and concentrations of furfural and hydroxymethylfurfural in untreated softwood hydrolysate and in the diluted solutions after overliming and activated carbon treatment.*

tively, and therefore also the amounts of inhibitory substances were diluted. Phenolic substances were analyzed by GC-MS from overlimed hydrolysates with 30 g l^{-1} sugar mass concentration. In straw hydrolysate coumaric acid (10 mg l^{-1}), vanillin (3 mg l^{-1}), and ferulic acid (4 mg l^{-1}) were detected, while softwood hydrolysate contained vanillin (9 mg l^{-1}) and benzoic acid (4 mg l^{-1}).

Fermentation of wood and straw hydrolysates

The differently treated straw hydrolysates all showed good fermentability, which can be seen in Figs. 7–9. Lactic acid yields based on the sum of glucose, xylose, and mannose are 90 % for the

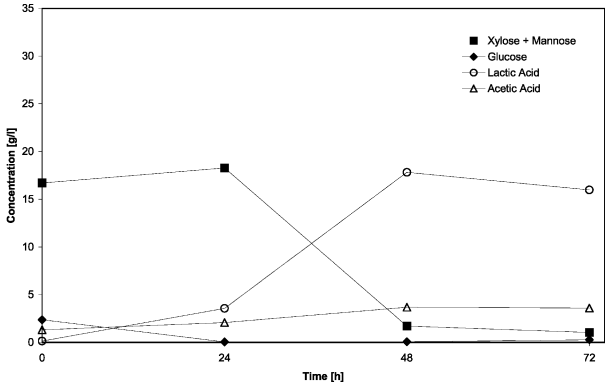

Fig. 7 – *Fermentation of straw hydrolysate containing approximately 15 g l^{-1} sugars with Bacillus BS121. The hydrolysate was pretreated by overliming.*

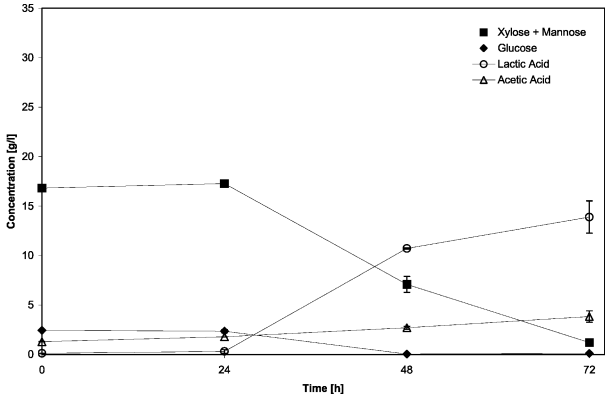

Fig. 8 – *Fermentation of straw hydrolysate containing approximately 15 g l^{-1} sugars with Bacillus BS121. The hydrolysate was pretreated by overliming and activated carbon treatment.*

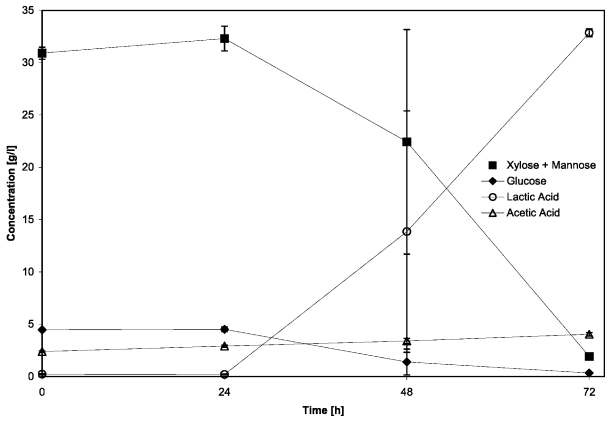

Fig. 9 – *Fermentation of straw hydrolysate containing approximately 30 g l^{-1} sugars with Bacillus BS121. The hydrolysate was pretreated by overliming and activated carbon treatment.*

overlimed hydrolysate with 15 g l^{-1} sugars, 77 % for the overlimed and activated carbon treated hydrolysate with 15 g l^{-1} sugars, and 99 % for the overlimed and activated carbon treated hydrolysate with 30 g l^{-1} sugars. Arabinose is not metabolized by the used strain and its concentration therefore remained constant. Acetic acid already present in the initial hydrolysates in low amounts, increases to concentrations of 3–4 g l^{-1}. In the 30 g l^{-1} experiment (Fig. 10) the results of the duplicates differ more than the usual range after 48 h, which is obviously due to an elongated lag-phase in one of the duplicates. However, after 72 h, when fermentation is finished, concentrations of lactic acid and sugars are identical in both samples.

Softwood hydrolysates showed a similar behavior regarding the sugar consumption after 48 h, however, lactic acid mass concentrations were considerably lower compared to the straw hydrolysate (Figs. 10–12). Lactic acid accounts for only 35, 38, and 23 % of the utilized sugars. Formation of acetic acid could not be observed either but acetaldehyde

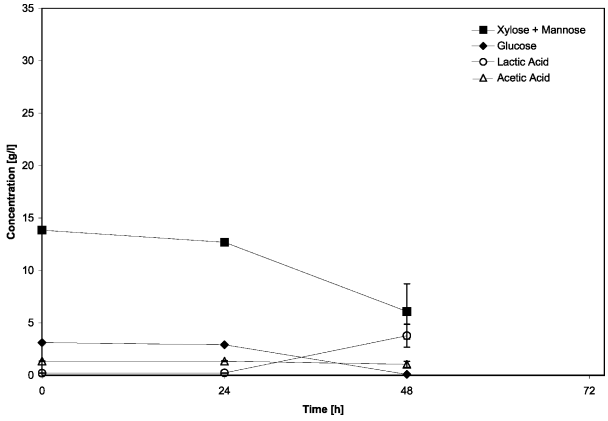

Fig. 10 – *Fermentation of softwood hydrolysate containing approximately 15 g l^{-1} sugars with Bacillus BS121. The hydrolysate was pretreated by overliming.*

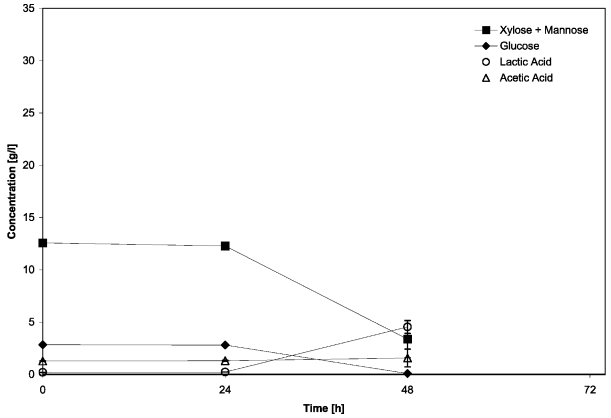

Fig. 11 – *Fermentation of softwood hydrolysate containing approximately 15 g l^{-1} sugars with Bacillus BS121. The hydrolysate was pretreated by overliming and activated carbon treatment.*

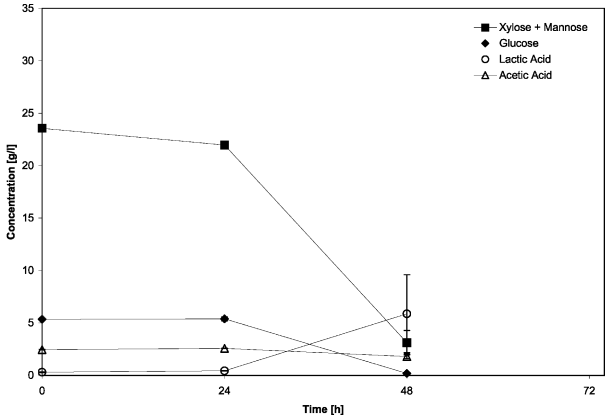

Fig. 12 – *Fermentation of softwood hydrolysate containing approximately 30 g l^{-1} sugars with Bacillus BS121. The hydrolysate was pretreated by overliming and activated carbon treatment.*

in concentrations similar to those of lactic acid was detected. The values for acetaldehyde show a high variation which is probably due to the fact that this compound is volatile at 60 °C.

Discussion

Dilute acid hydrolysis

The maximum sugar yield is 75 % of what could be expected after a complete hydrolysis of the hemicellulose fraction. Similar yields from softwood could be obtained by other researchers using acid hydrolysis or steam pretreatment.[17,18] The optimal reaction conditions resemble those that have been obtained for sugar cane bagasse with the same equipment.[13] High temperatures and long reaction times should be avoided, because they cause excessive formation of sugar degradation products. Favorable conditions are high acid concentrations (> 0.06 mol l^{-1}) at moderate temperatures (160 °C). Too high acid concentrations should be avoided, because more $Ca(OH)_2$ is needed for neutralization and higher amounts of $CaSO_4$ are generated by precipitation. This has a negative impact on the process economics and also questions the environmental benefits of the process in case of gypsum, which has to be considered as a waste product of the process.

Detoxification

Direct fermentation of dilute acid hydrolysates is not possible. In any case a neutralization is required, since pH values are usually below 2. The use of lime is recommended, since it leads to the precipitation of the sulfate and reduces the osmotic pressure in the solution. Neutralization with NaOH or KOH was observed to cause growth inhibition due to osmotic effects (data not published). Over-

liming is supposed to lead to the precipitation of inhibitors at the high pH values. It could be demonstrated that this method is effective in the removal of the sugar degradation products furfural and 5-hydroxymethylfurfural. Inhibition tests with a similar strain have shown, that 5-hydroxymethylfurfural can even promote the growth of microorganisms at concentrations below 0.2 g l^{-1}, and that phenolic compounds, which are generated by lignin degradation during the hydrolysis process lead to growth inhibition at much lower concentrations than sugar degradation products.[19] The high inhibitory effects of phenolic substances to microbial growth has also been observed by other researchers.[14,15,20] A concentration reduction of these substances was achieved by the activated carbon treatment, which does not only remove phenolics but also other inhibitory substances. Other methods that were suggested for the removal of phenolic substances are ion-exchange resins[15] and enzymes.[19] All these methods have in common that they are rather cost intensive and not always practical for large scale application. Only recently we developed a new method using non-activated wood charcoal for detoxification, which showed good results on softwood hydrolysates.[11] The advantage of this method is that the agent for detoxification can be produced at a low price using the same raw material that is used for the production of the hydrolysates.

Lactic acid fermentation

All fermentation experiments showed lactic acid formation from hemicellulosic sugars. In most experiments the lag-phase is longer than 24 h. The consumption of glucose during the first day in the overlimed straw hydrolysate with 15 g l^{-1} sugar mass concentration indicates a diauxic growth. Since the inoculum was grown on xylose, probably some time for adaptation is necessary in order to convert the hexoses. Lactic acid yields were considerably higher from straw than from softwood hydrolysates, which is remarkable, because straw hydrolysates contain considerably more xylose. Sugars from softwood hydrolysate are only partly converted into lactic acid and acetaldehyde is presumably formed as a second metabolite. The product formation is independent from the detoxification method and the metabolic pathway for the acetaldehyde formation remains unclear.

These results demonstrate that the production of lactic acid from lignocellulose hydrolysates using thermophilic *Bacillus* strains is basically possible, but the actual yields depend more on the type of the lignocellulosic substrate than on hydrolysis conditions, detoxification steps, or sugar concentrations. The use of *Bacillus* sp. for lactic acid production has been described earlier.[21,22] Although high yields and low by-product formation could be observed, the major problem remains the sensitivity of these strains to higher concentrations of lactic acid and therefore lactic acid mass concentrations higher than 60 g l^{-1} have not been described so far. The same phenomenon was observed with BS121.[23] However, these strains show many advantages including the fermentability of xylose, production of L-lactic acid with high optical purity, and low susceptibility to contamination.[24] Increased productivities can be obtained applying continuous fermentation with cell recycle in a membrane bioreactor. A system using a membrane bioreactor coupled with monopolar electrodialysis was used with a similar strain, *Bacillus* BS119, on glucose.[25] The advantage of this system is that the product is purified, while unused substrate, including sugars and other nutrients, can be recycled into the bioreactor.

List of symbols

c – concentration, mol l^{-1}
Q – volume flow rate, ml min^{-1}
R^2 – square corelation
t – time, min
T – temperature, °C
w – mass fraction, %
γ – mass concentration, g l^{-1}
φ – volume fraction,

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