Municipal green waste as feedstock for microbial fermentations

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Abstract

In view of the depletion of fossil fuels as well as an increasing demand of energy and resources, the need for a circular economy is evident. Material formerly seen as waste is considered as potential resource. In 2019, 5,194 t of municipal green waste accumulated in Germany [1], constituting a major waste stream in urban areas. Composting or the production of biogas, which is the method of choice for the deposal of this material so far, is economically not worthwhile. Municipal green waste comprises grass clippings as well as tree and hedge pruning from gardens, parks and cemeteries. Consisting mainly of lignocellulose, a polymer of lignin, hemicellulose and cellulose, municipal green waste is a promising feedstock for microbial fermentations. Besides carbohydrates, additional components such as proteins, vitamins and minerals can also be obtained from green waste. The complex structure of lignocellulose requires different pretreatment methods. Depending on the moisture content of the biomass, pressing leads to a nutritious press juice, which serves as fermentation medium or medium supplement for microbial cultures. Dry biomass and biomass containing elevated amounts of recalcitrant lignin is pretreated hydrothermally. The OrganoSolv process removes the latter using high temperatures and pressure as well as ethanol as solvent. Pretreated material is hydrolyzed enzymatically to release monosaccharides which are subsequently used as carbon source for fermentations.

Pretreatment

Pretreatment of lignocellulosic biomass is necessary to break up the polymeric structure of lignocellulose. The main goal is the removal of recalcitrant lignin and the dissolution of cellulose and hemicellulose. During a subsequent enzymatic hydrolysis, the carbohydrates are fragmented into monosaccharides.

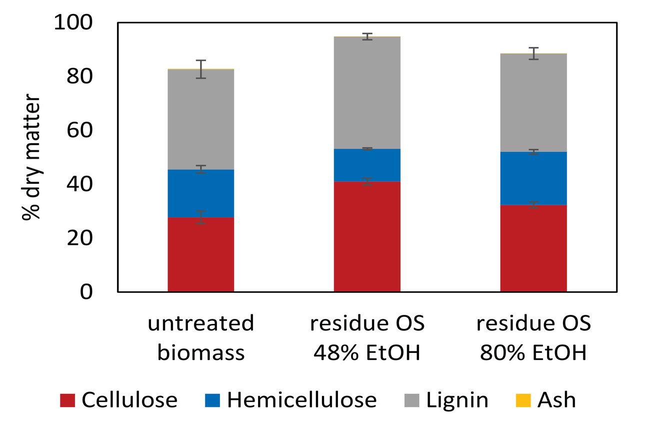


Fig 1: Composition of biomass prior to pretreatment and after hydrothermal pretreatment with different conditions. Biomass: wood shreds from beech and pine. Pretreatment conditions: OrganoSolv with 48% ethanol, 190°C, 78 min; OrganoSolv with 80% ethanol, 185°C, 15 min; n=2.

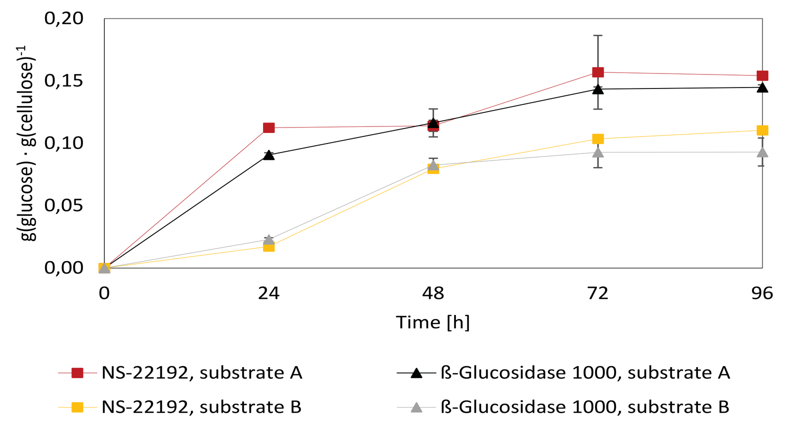
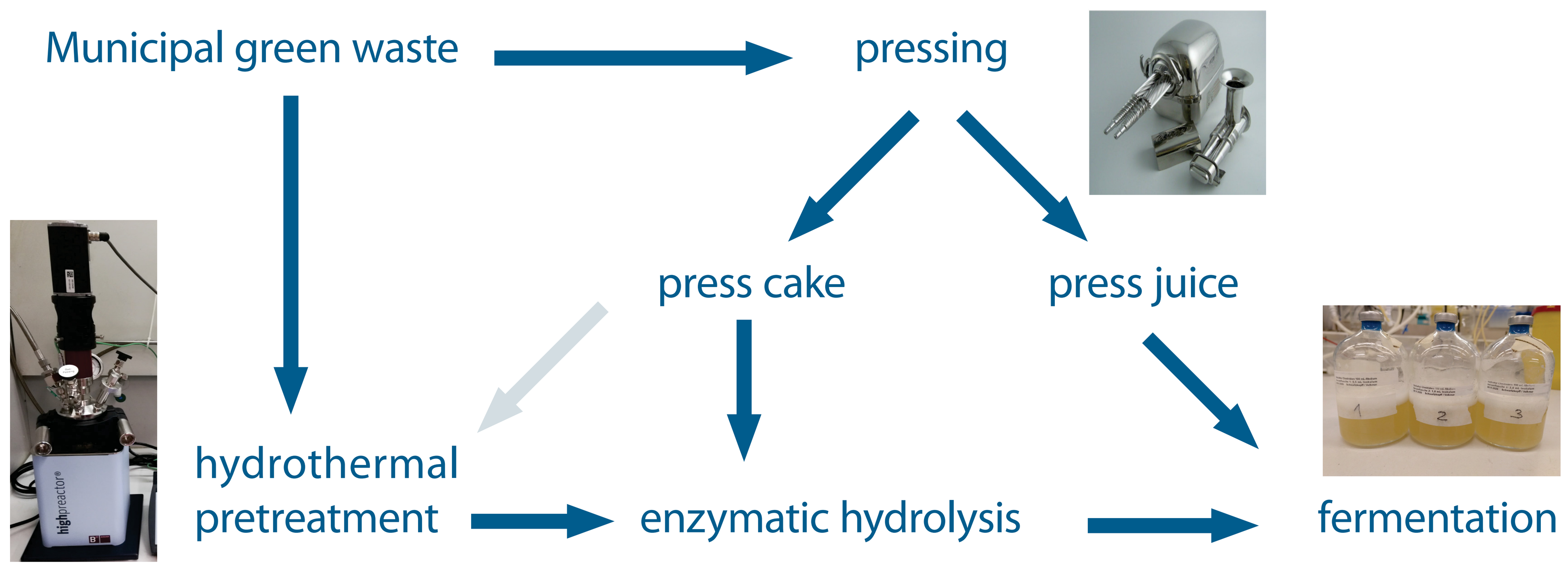


Fig 2: Enzymatic hydrolysis of lignocellulosic biomass with different enzyme mixtures. NS-22192, Novozymes, Denmark; ß-Glucosidase 1000, ASA Spezialenzyme, GmbH, Germany; both mixtures of ß-glucosidase, cellulases and hemicellulases. 2.8 mg NS-22192 per g Substrate, 2.5 mg ß-Glucosidase 1000 per g Substrate. Substrate A: residue from OrganoSolv pretreatment of wood shreds with 48% ethanol, substrate B: residue from OrganoSolv pretreatment of wood shreds with 80% ethanol. Hydrolysis at 30°C, overhead shaker, 0.1 M acetic acid-sodium acetate buffer pH 5.0 with 0.2 g·L^{-1} NaN_3; n=2.

Process schematic



Municipal green waste with a high moisture content is fractionated into a solid and liquid fraction using a screw press. The press juice can be used directly for microbial fermentations as fermentation medium or medium supplement. Dry biomass is pretreated hydrothermally in a high-pressure reactor using elevated temperature, pressure and ethanol as solvent. The residue as well as the solid fraction from the pressing step is hydrolysed enzymatically. As the strain during the pressing step constitutes a mechanical pretreatment, a further hydrothermal treatment of herbaceous biomass is not profitable.

Application of grass press juice in microbial fermentations

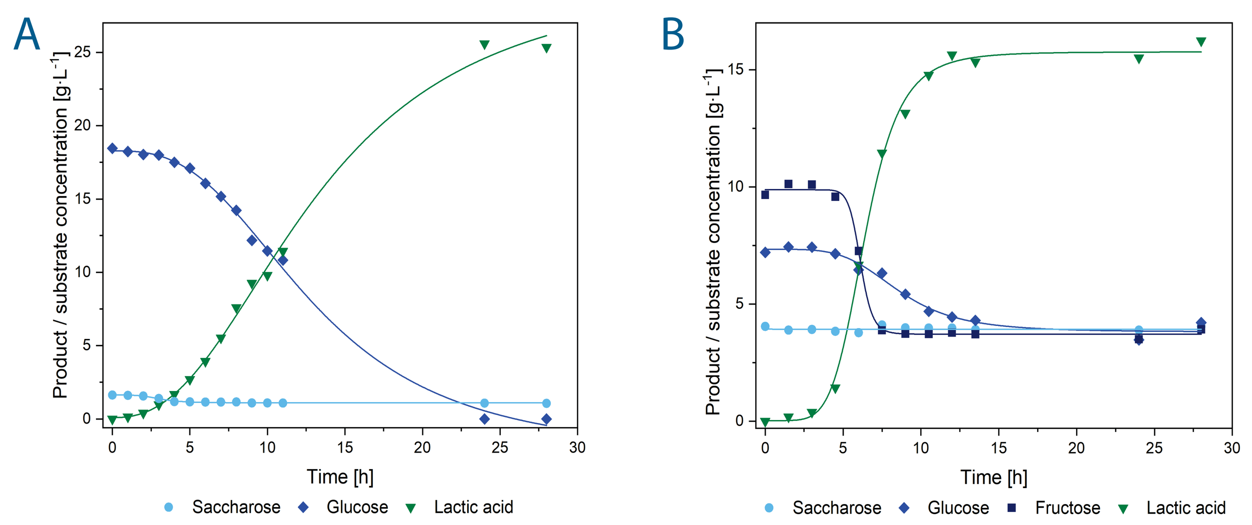


Table 1: Yield and productivity of fermentation of *Lactobacillus delbrueckii* subsp. *lactis* cultivated in MRS medium with $CaCO_3$ and press juice supplemented with MRS medium components and 0.1% invertase

	$Y_{P/S}$ in $g_{lactic\ acid} \cdot g_{sugar}^{-1}$	P_{24} in $g_{lactic\ acid} \cdot L^{-1} \cdot h^{-1}$	P_{max} in $g_{lactic\ acid} \cdot L^{-1} \cdot h^{-1}$
MRS + CaCO3	1.36	1.10	2.07
Press juice + MRS components + 0.1% invertase	1.58	0.65	3.50

Fig 3: Cultivation of *Lactobacillus delbrueckii* subsp. *lactis* in MRS medium with 0.6 $g_{CaCO3} \cdot g_{glucose}^{-1}$ for pH regulation (A) and autoclaved grass press juice supplemented with 25 g·L^{-1} MRS media components and 0.1% invertase (B).
Cultivation conditions: anaerobic conditions, 45°C, 120 rpm; MRS medium: casein-peptone 10 g·L^{-1}, meat extract 10 g·L^{-1}, yeast extract 5 g·L^{-1}, K_2HPO_4 2 g·L^{-1}, $C_2H_3NaO_2$ 5 g·L^{-1}, $C_6H_{17}N_3O_7$ 2 g·L^{-1}, $MgSO_4 \cdot 7\ H_2O$ 0.2 g·L^{-1}, $MnSO_4 \cdot H_2O$ 0.05 g·L^{-1},

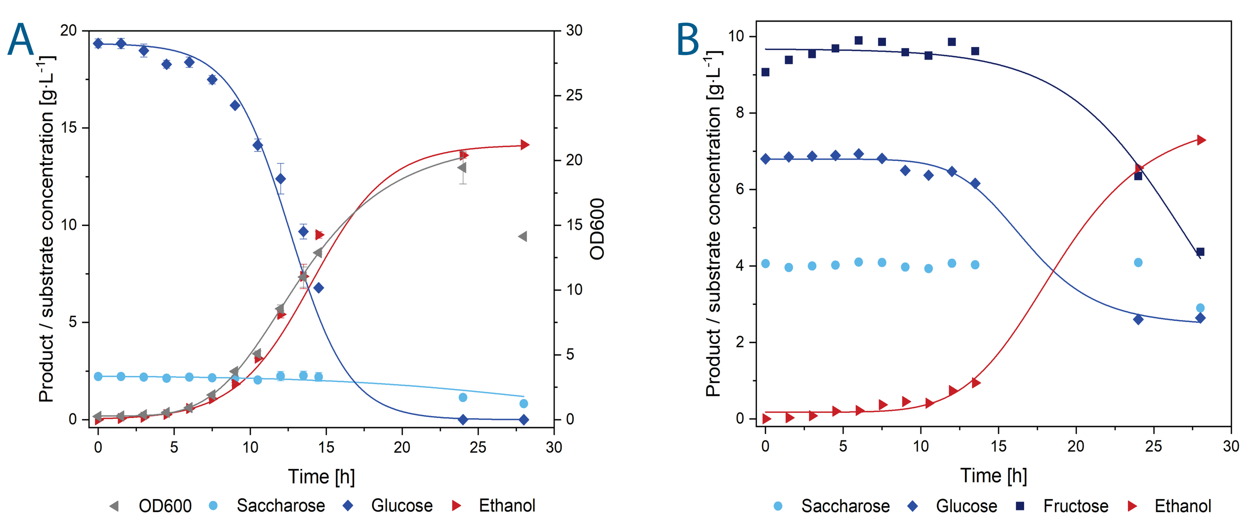


Table 2: Yield and productivity of fermentation of *Saccharomyces cerevisiae* in General Yeast Medium and autoclaved grass press juice supplemented with GYM components and 0.1% invertase

	$Y_{P/S}$ in $g_{ethanol} \cdot g_{sugar}^{-1}$	P_{24} in $g_{ethanol} \cdot L^{-1} \cdot h^{-1}$	P_{max} in $g_{ethanol} \cdot L^{-1} \cdot h^{-1}$
General Yeast Medium	0.70	0.57	2.14
Press juice + GYM components + 0.1% invertase	0.71	0.28	0.53

Fig 4: Cultivation of *Saccharomyces cerevisiae* in General Yeast Medium (A) and autoclaved grass press juice supplemented with General Yeast medium components and 0.1% invertase (B).
Cultivation conditions: anaerobic conditions, 32°C, 120 rpm; General Yeast medium: casein-peptone 5 g·L^{-1}, yeast extract 3 g·L^{-1}, malt extract 3 g·L^{-1}, glucose 20 g·L^{-1}; pH 6.5

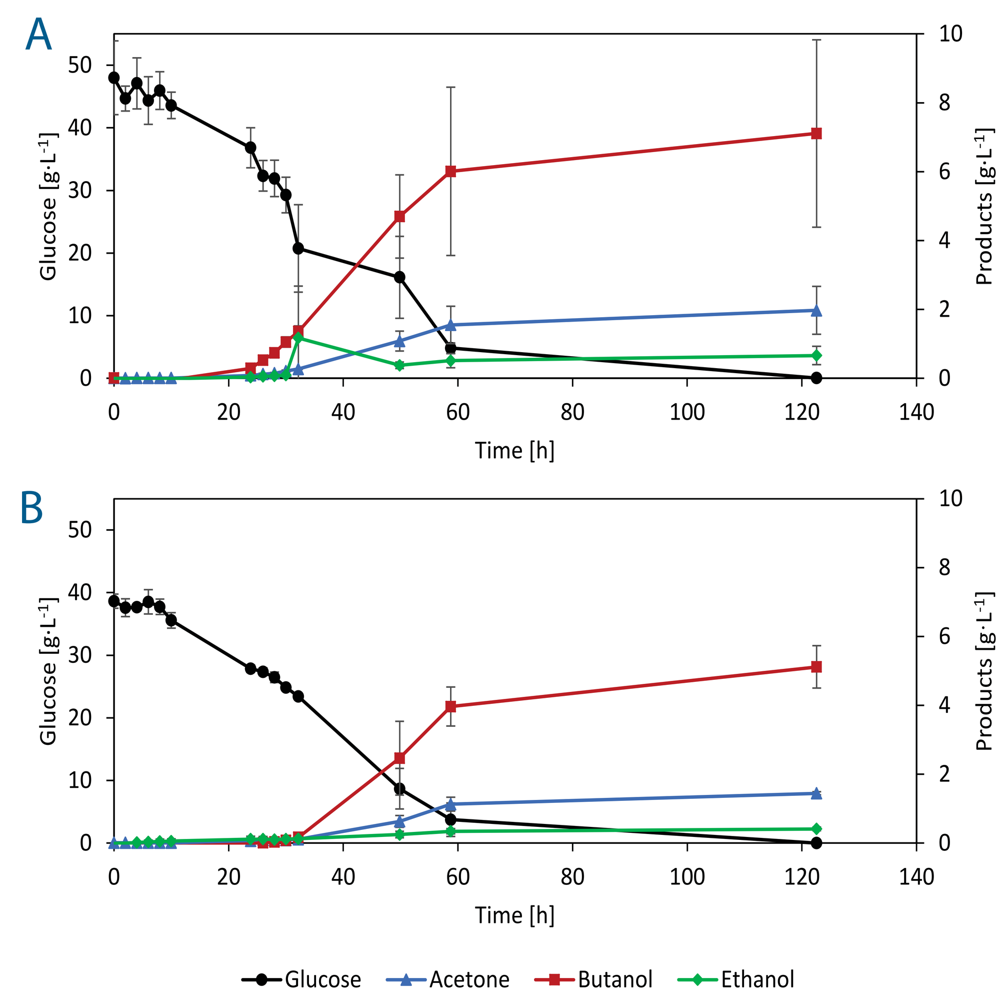


Fig 5: Cultivation of *Clostridium acetobutylicum* in standard medium (A) and standard medium supplemented with 5% sterile-filtered grass press juice (B). Cultivation conditions: anaerobic conditions, 37°C, 50 rpm; medium: D-glucose 45.00 g·L^{-1}, ammonium acetate 2.2 g·L^{-1}, KH_2PO_4 0.50 g·L^{-1}, K_2HPO 0.50 g·L^{-1}, $MgSO_4 \cdot 7\ H_2O$ 0.20 g·L^{-1}, Fe(II)$SO_4 \cdot 7\ H_2O$ 0.015 g·L^{-1}, $MnSO_4 \cdot H_2O$ 0.010 g·L^{-1}, *p*-aminobenzoic acid 2.00 mg·L^{-1}, D-biotin 0.01 mg·L^{-1}, thiamin-HCl 2.00 mg·L^{-1}. n=3.

Conclusion and perspective

A process schematic for the use of municipal green waste as feedstock for the fermentative production of base and fine chemicals was developed. Humid green waste was pressed using a screw press to obtain a press juice. Simultaneously, the biomass was mechanically pretreated, which supersedes further hydrothermal pretreatment. Hydrothermal pretreatment was performed with ethanol to remove recalcitrant lignin. Grass press juice is a suitable fermentation medium for the production of lactic acid by *Lactobacillus delbrueckii* subsp. *lactis* and ethanol by *Saccharomyces cerevisiae*. Furthermore, the suitability of the addition of grass press juice for the cultivation of *Clostridium acetobutylicum* was examined. Different enzyme mixtures were tested for the saccharification of municipal green waste. In a next step, the suitability of the enzymatic hydrolysate of biomass as carbon source for microbial fermentations is examined.

TECHNISCHE UNIVERSITÄT KAISERSLAUTERN

Literature

[1] Statistische Ämter des Bundes und der Länder, 2019, https://www.statistikportal.de/de/aufkommen-haushaltsabfaellen, accessed 26.04.2021

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http://www.mv.uni-kl.de/biovt

Financially supported by
Bundesministerium für Bildung und Forschung
Grant number 031B0903B