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Book of Abstracts

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chain non-competing carbon substrate. In addition we also investigated utilization of inexpensive waste-originating complex nitrogen sources such as proteolytic hydrolysate of cheese whey or alkaline hydrolysate of chicken feather which substantially improves process of PHA production by supporting growth of bacterial cultures but also by enhancing PHA accumulation in bacterial cells. Furthermore, we also aimed at valorization of low-cost lignocellulose materials such as spent coffee grounds or waste wood biomass employing biorefinery concept in which PHA production represent one of the key processes. For instance, spent coffee grounds can be completely utilized in sequential process, in which oil can be extracted from spent coffee grounds and used for PHA production employing Cupriavidus necator. Further, the solid residues after oil extraction can be hydrolysed yielding fermentable sugars, which are further used as a substrate for production of PHAs employing *Burkholderia cepacia*. Finally, solids after SCG hydrolysis possess high calorific value and can be used as a fuel to at least partially cover energetic demands of the process.

Furthermore, utilization of waste substrates is usually associated with non-optimal cultivation conditions induced by presence of various microbial inhibitors. Therefore, we also focused on influence of stress conditions on PHA production and, oppositely, also on involvement of PHA in stress resistance of bacteria. It is very interesting that numerous stress conditions usually connected with waste substrates such as lignocellulose hydrolysates (e.g. osmotic pressure, oxidative pressure, presence of weak organic acids etc.) stimulates PHA biosynthesis in bacterial cells when applied at mild level. Oppositely, presence of PHA in bacterial cells considerably enhances their resistance against numerous stress factors such as osmotic pressure, temperature or oxidative stress. Therefore, it seems that microbial production of PHA is very interesting process robust to specific conditions associated with utilization of complex and non-optimal substrates in the concept of bio-refinery.

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Bench-scale production of polyhydroxyalkanoates and other valuable biomaterials from xylose-rich lignocellulosic hydrolysates

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The set of work developed at Instituto Superior Técnico addressed the optimization of polyhydroxyalkanoates (PHA)

production from wheat straw lignocellulosic hydrolysates (WSH). PHAs are biodegradable and bioproduced polymers, suitable for applications in fields such as agriculture, food packaging, medicine and pharmacy[1,2]. In most PHA producing bacterial strains, poly-3-hydroxybutyrate (P(3HB)) accumulation as carbon and energy storage granules is favored by an excess carbon source and a low supply of macronutrients (N, P, O_2) or micronutrients (e.g. Mg). A range of different C-sources is metabolized to produce PHA co- and terpolymers. Research efforts have been devoted to trying to decrease production and extraction costs in order to increase the market share of these polymers[2].

Burkholderia sacchari DSM 17165 was chosen due to its simultaneous ability (i) to metabolize C-5 and C-6 sugars and (ii) to produce PHA. Polymer accumulation was triggered by P-limitation. At *biorefinery.de*, lignocellulosic biomass (chopped wheat straw) was pretreated using the AFEX process followed by enzymatic hydrolysis and a concentration step, originating WSH with different glucose/xylose ratios. C-sources were quantified by HPLC, PHA and gamma-butyrolactone (GBL) by GC[3,4]. For PHA recovery from lyophilized cells, solvent extraction was followed by precipitation with C_2H_5OH[5]. Fed-batch experiments were run on 2L bench scale stirred tank bioreactors, under controlled conditions with on-line data acquisition.

Assays ran with real WSH were compared to "simulated"hydrolysates with different glucose/xylose ratios. Feedback from bench-scale assays allowed for WSH improvement (sugars:organic acids: inhibitors ratio) by *biorefinery.de*. Remarkable P(3HB) volumetric productivities ($Prod_v ol$) of 1.7 g/L*h were obtained for P(3HB) production from WSH as sole C-source. These high productivities resulted from process optimization, involving the choice of the strain and cultivation media, and fed-batch operating conditions. Cell density and P(3HB) $Prod_v ol$ obtained were similar to those reached in control cultivations with mixtures of commercial sugars. Additionally, fed-batch strategies for the production of P(3HB-co-4HB) on glucose and GBL were developed, and led to copolymer accumulation with $Prod_v ol$ reaching 0.5 g/L*h using wheat straw hydrolysates as major carbon source[6].

Recently, the authors reported for the first time that B. sacchari DSM 17165 is also able to produce xylitol and xylonic acid from low glucose/xylose ratio mixtures[7]. Further optimization is under way to find the best culture conditions to favor either PHA, xylitol or xylonic acid production from xylose rich hydrolysates, towards an integrated biorefinery concept.

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Consolidated production of Volatile Fatty Acids from plant biomass using defined and natural microbial consortia

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A process design approach for consolidated production of Volatile Fatty Acids (VFAs) from plant biomass was developed. The design of the reactor enables simultaneous aerobic and anaerobic conditions in direct physical closeness and thus allow different microorganisms to coexist, grow and co-operate. Both defined synthetic microbial consortia as well as natural undefined ones were used to hydrolyze the plant biomass and convert the released sugars to the desired fatty acids. The production can be directed towards different carboxylic acids by either selecting the microorganisms in the case of defined consortia or by controlling the process conditions (in the case of natural ones).

A synthetic fungal-bacterial consortium for the direct production of lactic acid from cellulosic biomass was developed. The aerobic fungus *Trichoderma reesei* was introduced as producer of cellulolytic enzymes and the facultative anaerobic bacterium *Lactobacillus pentosus* was used as the product forming microorganism. The cellulolytic activity of the system was investigated and the addition of *Aspergillus niger* as a second enzymes producer was evaluated.

The use of natural microbial consortia for the production of VFAs was also studied. We examined the ability of our system to provide suitable growth conditions to different microbial members of the consortium, for the direct production of VFAs from biomass. Furthermore, we studied the effect of the introduction of a cellulolytic enzyme producer, on VFAs yields and productivities.

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Comprehensive Characterization of Lignin and its Degradation Products: Approaches and Challenges

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