An integrated process for the valorization of sugar streams after organosolv pretreatment of lignocelluloses towards the production of nutraceuticals and polymer building blocks

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The valorization of biomass residues towards the production of value-added products is attracting increasing interest during the last years, not only because lignocellulose is a renewable source of sugars that can be transformed into chemicals platform for different applications, but also because it is an abundant waste stream, most of the times under-valorized. ***Lactic acid*** is an important bio-product due to its use in biodegradable and biocompatible poly-lactate polymers (PLA). Blending poly L-lactic acid with poly D-lactic acid greatly improves the mechanical and physical properties of the material, therefore the production of D-lactic acid has attracted significant attention (Klotz *et al.,* 2016). Lactic acid bioconversion processes have numerous advantages over the chemical synthesis route, not only due to the high-titer yield of the final product with great optical purity. ***Poly-unsaturated fatty acids (PUFAs)***, such as eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA), are widely recognized as important bioactive compounds used in the food and nutraceutical industry for the development of functional foods with scientifically sustained claims (Kahn *et al*., 2013). Omega-3 fatty acids fortified products, together with prebiotics and probiotics enriched food formulations, belong to top market investment pockets that have attracted the interest of consumers (Ganesan *et al*., 2014). Microalgae are oleaginous microorganisms that are able to accumulate high amounts of omega-3 fatty acids when growing in heterotrophic cultures, supported by a carbon source, offering an attractive alternative to the fish oil which, minimizing the adverse effects from the unpleasant odor and the presence of contaminants, like dioxins, and heavy metals. ***Xylo-oligosaccharides*** are compounds with great prebiotic potential that can be incorporated into many food products (Christakopoulos et al., 2003) and mainly derive from hemicellulose xylan-backbone degradation.

For the proposed integrated process (Figure 1), we employed a mild oxidative organosolv pretreatment method as an initial step, which offers great fractionation efficiency in the absence of any acid catalyst and results to low yields of inhibitor compounds. Screening of various pretreatment conditions, such as the type of the organic solvent used and the cooking time, occurred in order to optimize the subsequent enzymatic saccharification and fermentation yields. We utilized the pretreated cellulose-rich pulps to study the production of enantiomerically pure D-lactic acid with the obligately homofermentative lactic acid bacterium *Lactobacillus delbrueckii* subsp. *bulgaricus* (ATCC® 11842™) through simultaneous saccharification and fermentation (SSF). In parallel, we enzymatically treated the solid fractions with a commercially available cellulolytic cocktail, Cellic® CTec2, and tested the enzymatic hydrolysates for their ability to support the growth and lipid accumulation of marine heterotrophic microalgae *Crypthecodinium cohnii* in batch and fed-batch cultures. The liquid fraction, after the removal of organic solvent, was analyzed for the presence of prebiotic xylo-oligosaccharides.

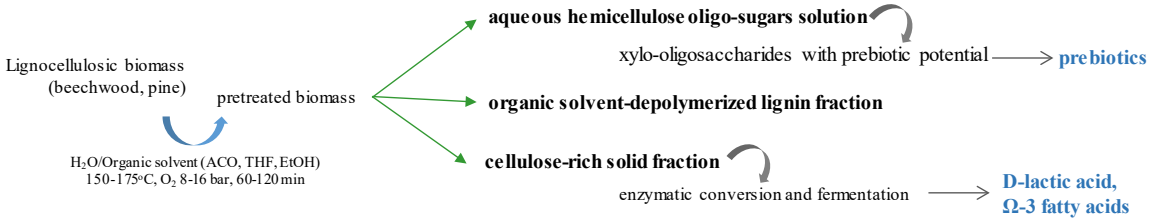

Figure 1. Overall process scheme of the integrated process for the valorization of cellulosic and hemicellulosic biomass fractions after pretreatment towards the production of value added products.

For the production of D-lactic acid, we employed SSF in batch cultures with 9% (w/v) solids loading, upon addition of 9 mg cellulases/ g of substrate and $CaCO_3$ to a level of 0.5 g/g glucan. The addition of $CaCO_3$ leads to the formation of calcium lactate, thus eliminating the end-product inhibition by undissociated lactic acid. The results showed that H_2O/EtOH and H_2O/ACO pretreated beechwood pulps at 160 and 175 °C for 120 min were the best candidates for lactic acid production with yields of 0.68-0.69 g/g of substrate. The highest production of 62 g/L lactic acid after 72 h of incubation was with H_2O/EtOH and corresponded to 82.7% of the theoretical

maximum yield and a productivity of 0.86 g/(L*h). For the untreated biomass, the produced lactic acid was 6.5 g/L that corresponded to 15.4 % of the theoretical maximum yield, a volumetric productivity of 0.09 g/L/h and yield of 0.07 g/g biomass. All the pretreated materials showed higher lactic acid yield, verifying the importance of the pretreatment step in biomass valorization. Our results showed that pulps with >57 wt. % cellulose, reaching up to 87% wt. were obtained, which were subsequently used as carbon sources without any detoxification to achieve high yields of D-lactic acid.

For the *C. cohnii* cultivations, the cellulose-rich pulps after pretreatment were enzymatically treated at 9% (w/v) solids loading, upon addition of 9 mg cellulases/ g of substrate, for 72 h. Hydrolysates were diluted 2-times, so as the initial sugar content to be approximately 30 g/L in order to eliminate the inhibitory effect of high glucose concentration on growth of microalgal cells. All different beechwood pulps could efficiently support the growth of microalgae and the synthesis of fatty acids, with DHA ranging from 26.6 to 29.5 wt. % of the total lipids, as summarized in Table 1, while DHA reached up to 43.5% of the cell's total lipids. In order to increase the accumulation of lipids and optimize the production of DHA by applying nitrogen limitation conditions, a fed-batch strategy was employed. This approach led to an increase in the final biomass yield, but also to a reduction in wt. % fatty acids, indicating that optimum growth and maximum DHA accumulation by the microalgae cells require different medium compositions.

Analysis of the aqueous pretreatment liquid fraction showed the direct formation of xylo-oligosaccharides up to a concentration of 17 g/L (biomass treated with H_2O/EtOH (50/50%), O_2 16 bar, 160ºC, 120min), without applying any additional enzymatic treatment. The oligo-sugars were tested as fermentative substrates for probiotic *Lactobacilli* bacteria in order to verifying their prebiotic potential. Our results demonstrate the utilization of lignocellulosic biomass in an economic and environmentally friendly way to produce value-added products.

Table 1. Biomass and total fatty acid (TFA) production by *C. cohnii* cells in batch cultivation. *fed:* refers to fed-batch cultures. % TFA refers to the amount of total lipids out of the total biomass on a weight basis.

Pretreatment	Biomass (g/L)	%TFA	TFA (g/L)	% DHA	DHA (g/L)
H_2O/ACO, O_2 12 bar, 175ºC, 120min	6.5 *(0.1)*	45.9 *(1.0)*	3.0 *(0.0)*	27.7 *(2.0)*	0.8 *(0.0)*
H_2O/EtOH , O_2 12 bar, 175ºC, 120min	8.6 *(0.4)*	54.8 *(1.3)*	4.7 *(0.0)*	28.6 *(3.2)*	1.4 *(0.2)*
H_2O/THF, O_2 12 bar, 175ºC, 120min	7.4 *(0.4)*	44.2 *(1.1)*	3.3 *(0.4)*	27.2 *(1.5)*	0.9 *(0.1)*
H_2O/ACO, O_2 12 bar, 175ºC, 60min	7.8 *(0.2)*	33.5 *(0.6)*	2.6 *(0.2)*	29.0 *(0.5)*	0.8 *(0.0)*
H_2O/EtOH, O_2 12 bar, 175ºC, 60min	8.7 *(0.0)*	39.1 *(1.0)*	3.4 *(0.0)*	28.2 *(2.0)*	1.0 *(0.2)*
H_2O/THF, O_2 12 bar, 175ºC, 60min	8.0 *(0.1)*	35.7 *(1.1)*	2.9 *(0.3)*	29.5 *(0.9)*	0.8 *(0.0)*
H_2O/ACO, O_2 16 bar, 175ºC, 120min	7.7 *(0.7)*	38.2 *(2.1)*	3.0 *(0.4)*	22.0 *(3.2)*	0.7 *(0.1)*
H_2O/EtOH, O_2 16 bar, 175ºC, 60min	8.6 *(0.2)*	48.0 *(0.2)*	4.1 *(0.3)*	29.5 *(2.1)*	1.2 *(0.3)*
H_2O/THF, O_2 16 bar, 150ºC, 120min	7.9 *(0.2)*	54.3 *(0.3)*	4.3 *(0.2)*	29.4 *(1.4)*	1.3 *(0.1)*
untreated beechwood	1.0 *(0.1)*	36.0 *(1.7)*	0.4 *(0.1)*	14.7 *(3.4)*	0.1 *(0.0)*
H_2O/EtOH, 16 bar, 175ºC, 60min ***(fed)***	12.7 *(0.4)*	44.9 *(0.7)*	5.7 *(0.2)*	38.7 *(0.5)*	2.2 *(0.1)*
H_2O/THF, 16 bar, 150ºC, 120min ***(fed)***	10.3 *(0.1)*	38.5 *(0.9)*	4.0 *(0.0)*	43.5 *(0.2)*	1.7 *(0.2)*

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