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Hemp hurds biorefining: A path to green L-(+)-lactic acid production

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HIGHLIGHTS

- Hemp hurds were subjected to mild organosolv pretreatment and enzymatic hydrolysis.
- High yields of fermentable carbohydrates (C5 and C6) and lignin were obtained.
- No detoxification step before fermentation of sugar was required.
- Lignocellulosic sugars were fermented to L-lactic acid by *Bacillus coagulans* XZL4.
- Lactic acid titers were 109 g/L from C5 and 141 g/L from C6.

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ABSTRACT

Sugars streams generated by organosolv pretreatment of hemp hurds, cellulose (C6) and hemicellulose (C5) fractions, were fermented to lactic acid (LA) by *Bacillus coagulans* strains XZL4 and DSM1. Pretreatment conditions and enzymatic hydrolysis were optimized and *B. coagulans* aptness to use lignocellulosic-derived sugars as a carbon source was evaluated. Methanolic organosolv pretreatment with 2.5% (w/w) H_2SO_4 gave the best results in terms of glucan recovery (98%), enzymatic hydrolysis of pretreated biomass (70%) and hemicellulosic sugars recovery (61%). C6 and C5 sugars fermentation by strain XZL4 gave, high LA yields (0.90 and 0.84 g/g), high titers (141 and 109 g/L), and high enantiomeric excess (>99%). Overall, 42 g of L-LA were obtained from 100 g of raw hemp hurds. These results can be considered promising for lignocellulosic feedstock valorization toward the production of polymer-grade LA.

1. Introduction

Replacement of current fossil oil-based economy with the so-called sugar platform route will require some breakthrough changes in the today's production of goods. Within this context, new synergies between biological and chemical sciences are required to exploit the potential of lignocellulosic biomasses (Cherubini, 2010). Industrial hemp (*Cannabis sativa* L.) is a fast growing and a high yielding annual crop that provides a wide range of valuable products. High land use efficiency as well as improvement of soil health are some of the main feature of this species, supporting its use also as an energy crop (Karus and Vogt, 2004). Hemp hurds are the industrial by-products from fiber production, which is currently discarded as solid waste. Due to the high content of carbohydrates (~70% dry weight), mostly glucose and xylose (Gandolfi et al., 2013), hemp hurds can be considered a potential source of inexpensive fermentable sugars. Aim of the biorefinery approach is to depolymerize and deoxygenate all the biomass components, that is cellulose, hemicellulose and lignin, to obtain a number of platform chemicals via fermentations or chemical synthesis. However, due to the compact and rigid structure of lignocellulosic materials, known as biomass recalcitrance, the release of fermentable sugars has become a bottleneck for industrialization of lignocellulosic biorefinery. In this respect, considerable research has been carried out to enhance cellulolytic enzymes performance and several factors (e.g., lignin content,

Abbreviations: AIL, acid insoluble lignin; ASL, acid soluble lignin; BCA, bicinchoninic acid assay; CS, combined severity; FPU, filter paper unit; HMF, 5-hydroxymethylfurfural; LA, lactic acid; OS, organosolv; OSL, organsosolv lignin; PLA, poly-lactic acid.

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accessible surface area, pore volume and cellulose crystallinity) are considered to determine the saccharification rate, thus necessitating the use of a pretreatment step prior to enzymatic hydrolysis (Zhao et al., 2012). Even though a variety of pretreatment protocols have been reviewed (Galbe and Zacchi, 2012), the selective fractionation of all lignocellulosic components still remains one of the main open issues in biorefinery. Among them, the OS process can be considered the preferred one to obtain simultaneously valuable hemicellulosic monomers, low-crystalline cellulose and unaltered pure lignin (Zhao et al., 2009). Even though OS is a more expensive technology, process variables optimization and use of recyclable solvents would reduce the overall process cost and abide by the green process principles. Specifically, low boiling points solvents (e.g., methanol, ethanol and acetone) are the most used. OS can be performed with or without catalyst additions, in the latter case temperature over 180 °C should be reached to generate organic acids, from the hemicellulose hydrolysis, which acts as catalyst for the process (Zhao et al., 2009). However the addition of mineral acids (e.g., sulfuric, hydrochloric and phosphoric acid) as well as organic acids, has been proved to enhance the delignification process and hemicellulose dissolution, leading to a pretreated biomass that can be easily hydrolyzed by cellulolytic enzymes (Del Rio et al., 2010). OS is commonly considered a flexible pretreatment technology and different target chemicals, such as furan-type compounds, organic acids or bio-oils, can be obtained by changing the process harshness (Wettstein et al., 2012).

Beyond conversion of sugar to furans and organic acids, fermentation of carbohydrates can lead to wide range of products. Among them, LA is a versatile chemical used in food, cosmetic, pharmaceutical, textile and chemical industries, which could be produced by fermentation of lignocellulosic-derived sugars. Over the past few years, its application has been extended also to biodegradable plastics, to synthesize PLA polymers (Abdel-Rahman et al., 2013). The properties of PLA are similar to those of petroleum-derived polymers, so that it can replace them in several instances (e.g., packaging, fiber and foam materials). Because the physical properties of PLA depend on the isomeric composition of its monomers, production of enantiomerically pure LA is highly desirable. Currently, LA is obtained on an industrial scale by fermentation of pure sugars or edible crops by lactic acid bacteria (LAB), which typically have complex nutritional requirements (Wee et al., 2006).

Bacillus coagulans, a homolactic bacterium, has been reported to possess many valuable fermentation features, such as thermophilic trait, simple nutrition requirements and high carbon-efficiency (Patel et al., 2006). Moreover, it produces enantiomerically pure LA. To improve the economy of LA production, different authors have proposed the use of low-cost and renewable raw materials as carbon source for fermentations, such as corncomb molasses (Wang et al., 2010) and paper sludge (Budhavaram and Fan, 2009). In the present study, the feasibility of L-(+)-LA production at high concentration, from hemp hurds was investigated. To this aim, the H_2SO_4 concentration for OS fractionation of hemp hurds and the enzyme and solid loadings for the enzymatic cellulose hydrolysis, were optimized toward the recovery of fermentable sugars (C5 and C6). Moreover, best *B. coagulans* strains XZL4 and DSM1 fermentation conditions were investigated and an efficient non-sterile process for LA production was developed, using the hemp hurds-derived sugars streams.

2. Methods

2.1. Raw materials

Hemp hurds was provided by Assocanapa (Carmagnola, Italy) as chopped pieces of 5 cm in length or shorter. Biomass was manually separated from dust and short fiber, knife milled under 2 mm screen (MF-10, IKA, Germany) and extracted first with CH_2Cl_2 and then with acetone using a 1 L Soxhlet apparatus (12 h each, 3–4 cycles/h).

The composition of hemp hurds was 44% cellulose, 23% lignin, 25% hemicellulose and 1.2% ash (Gandolfi et al., 2013). The enzymes blend CTec2 was generously provided by Novozymes (Bagsværd, Denmark). *B. coagulans* strain XZL4 was isolated by Prof. Xu (Shangai Jiao Tong University), while strain DSM1 was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany). Bacterial cells were stored in a 20% (v/v) glycerol stock solution at −80 °C. All chemicals were used as received and were of analytical grade.

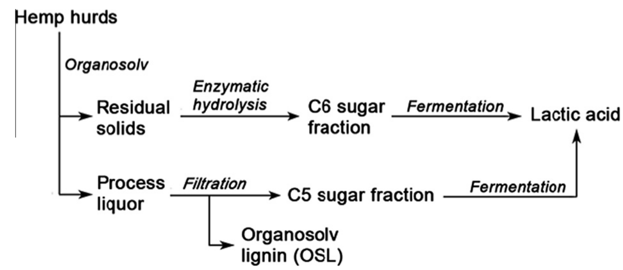

Fig. 1. Schematic overview of the experimental methodology.

Table 1
Compositional analysis of untreated and pretreated hemp hurds.

H_2SO_4 (%)[a]	CS	Solids[b] (%)	Glucan (%)	Xylan (%)	AIL (%)	ASL (%)
un.[c]	n.a.[d]	n.a.	44.5	23.3	25.5	2.0
0	−1.0	90.6	48.8	21.2	23.8	1.8
1	−0.3	82.3	52.9	17.9	21.9	2.2
2	0.5	68.4	63.3	10.6	17.9	1.5
2.5	1.3	59.1	73.7	9.4	13.0	0.9
3	1.7	43.9	73.0	5.9	9.6	1.1

[a] Based on biomass dry weight.
[b] Residual solids recovery.
[c] Untreated hemp hurds.
[d] Not applicable.

Table 2
Compositional analysis of OS process liquor.

CS	OSL (g)	Glu[a] (g/L)	Xyl[b] (g/L)	HMF (g/L)	Fur[c] (g/L)	LvA[d] (g/L)	AcA[e] (g/L)
−1.0	0.14	0.07	1.05	0.003	0.001	n.d.[f]	0.009
−0.3	0.19	0.30	6.82	0.008	0.003	0.009	0.014
0.5	0.62	1.94	14.48	0.017	0.137	0.037	0.169
1.3	1.26	2.73	16.14	0.019	0.141	0.058	0.163
1.7	1.48	3.63	19.63	0.154	0.163	0.281	0.357

[a] Glucose.
[b] Xylose.
[c] Furfural.
[d] Levulinic acid.
[e] Acetic acid.
[f] Not detected.

2.2. Organosolv pretreatment

The pretreatment of hemp hurds was performed in a 300 mL high-pressure reactor system (Berghof, BR-300, Germany) with temperature control (Berghof, model BTC-3000) and continuous stirring, as previously described (Gandolfi et al., 2014) The solids loading used in all the experiments was 10% (w/v). Hemp hurds (10 g) were soaked with a 65% (v/v) of a methanolic solution and with a content of concentrated sulfuric acid 0–3% (w/w, based on