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Evaluation of an integrated biorefinery based on fractionation of spent sulphite liquor for the production of an antioxidant-rich extract, lignosulphonates and succinic acid

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HIGHLIGHTS

- Two biorefinery schemes based on sulphite pulping mills were evaluated.
- Fractionation of SSL was achieved via nanofiltration and solvent extraction.
- An antioxidant-rich extract was produced via solvent extraction with ethyl acetate.
- Solvent extraction with isopropanol led to significant removal of heavy metals.
- 39 g/L succinic acid were produced from filtrated-extracted SSL.

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ABSTRACT

Spent sulphite liquor (SSL) has been used for the production of lignosulphonates (LS), antioxidants and bio-based succinic acid. Solvent extraction of SSL with isopropanol led to the separation of approximately 80% of the total LS content, whereas the fermentations carried out using the pretreated SSL with isopropanol led to the production of around 19 g/L of succinic acid by both *Actinobacillus succinogenes* and *Basfia succiniciproducens*. Fractionation of SSL via nanofiltration to separate the LS and solvent extraction using ethyl acetate to separate the phenolic compounds produced a detoxified sugar-rich stream that led to the production of 39 g/L of succinic acid by *B. succiniciproducens*. This fractionation scheme resulted also in the production of 32.4 g LS and 1.15 g phenolic-rich extract per 100 g of SSL. Both pretreatment schemes removed significant quantities of metals and heavy metals. This novel biorefinery concept could be integrated in acidic sulphite pulping mills.

1. Introduction

The production of bio-based polymers and chemicals is dependent on the efficient utilization of lignocellulosic biomass as well as industrial waste and by-product streams (Mussatto and Roberto, 2004; Koutinas et al., 2014). Lignocellulosic biomass is considered to be the most abundant feedstock on earth, representing approximately 50% of global biomass (Fernandes et al., 2011). The potential use of lignocellulosic biomass in fermentation processes is feasible after its conversion to monomeric sugars. An important source of fractionated lignocellulosic biomass is the waste stream from the wood pulp and paper industry. The spent sulphite liquor (SSL), the by-product of the acidic sulphite wood pulping process, is rich in C5 (xylose, arabinose) and C6 (glucose, mannose, galactose) sugars and its use for the production of bioethanol has already been studied (Marques et al., 2009; Pereira et al., 2013).

During the sulphite pulping process, lignin is removed from the wood under acidic conditions (pH 1–2) at 135–145 °C for 8–12 h in batch digesters using aqueous solution of $SO_2/MHSO_3/MSO_3$ (M stands for Na, Ca, Mg or NH_3) (Marques et al., 2009). Hardwoods, such as beech, eucalyptus and birch, are most commonly used (Pereira et al., 2013). During this process, lignin is fractionated and removed from the pulp as salts of lignosulphonic acid along with sugars derived from the hydrolysis of hemicelluloses. Lignosulphonates and sugars, with xylose being the predominant one, are the major compounds in SSL. This waste contains also sugar

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degradation products such as furfural, 5-(hydroxymethyl)furfural and acetic acid as well as phenolic compounds derived mainly from lignin degradation. Some of these compounds inhibit microbial growth hindering the direct valorization of SSL via microbial fermentation (Alexandri et al., 2016).

Several detoxification methods of lignocellulosic biomass have been studied including treatment with activated carbon, overliming, ethyl acetate extraction, evaporation and biological treatment with fungi (Pereira et al., 2013). Among the inhibitory compounds, phenolics are of major importance because their removal from the SSL could result not only to a less toxic fermentation substrate but also to a potential commercial product (Alexandri et al., 2016). Lignosulphonates (LS) are also of major importance as they are already used in various applications (e.g. concrete additives, plasticisers, cement dispersants). Nevertheless, the current process used for the recovery of LS from the SSL, based on calcium or sodium hydroxide treatment, leads to the destruction of sugars and therefore an unsuitable substrate for microbial fermentation.

Succinic acid (SA) is one of the most important bio-based platform chemicals that could be produced via fermentation (Taylor et al., 2015). Several literature-cited studies have focused on the production of succinic acid by microbial fermentation using renewable resources, such as corn stover, pinewood extract, sugarcane bagasse, spruce and wheat milling by-products (Hodge et al., 2009; Dorado et al., 2009; Xi et al., 2013; Wang et al., 2014; Salvachua et al., 2016). The bacterial strain *Actinobacillus succinogenes* is the most widely studied microorganism for succinic acid production (Koutinas et al., 2014), while *Basfia succiniciproducens* has been recently isolated and presents potential for succinic acid production (Becker et al., 2013). Alexandri et al. (2016) has carried out preliminary batch fermentations using either 7 times diluted untreated SSL or pretreated SSL via solvent extraction to remove only phenolic compounds showing that the latter medium improves succinic acid production mainly by *B. succiniciproducens*.

The sustainable conversion of acidic sulphite wood pulp mills into integrated biorefineries necessitates the valorization of SSL through the utilization of all components for the production of value-added products. Within this context, the aim of this study is to evaluate two different biorefinery strategies. In the first biorefinery concept, solvent extraction of SSL is employed for the production of LS and succinic acid. In the second biorefinery concept, nanofiltration of the SSL is followed by solvent extraction of the permeate leading to the production of LS, phenolic compounds and succinic acid. The detoxification of SSL was combined with the separation of value-added products leading also to improved production of succinic acid.

2. Materials and methods

2.1. Raw material

The SSL used in this study was provided by the company Sniace S.A. (Torrelavega, Spain) and was produced by the acidic sulphite pulping process of hardwood *Eucalyptus globulus* using dolomite. The SSL contained 176.5 ± 4.85 g/L total sugars (128.1 ± 0.6 g/L xylose, 21.5 ± 2.5 g/L galactose, 19.3 ± 0.4 g/L glucose, 7.4 ± 1.3 g/L mannose and 0.2 ± 0.05 g/L arabinose), 458.8 ± 2.7 g/L LS, 12.4 ± 0.8 g/L phenolics, pH value of 2.7 and 64 ± 0.2% dry matter. All results are average values of triplicate samples.

Two different biorefinery strategies were evaluated in this study. The first biorefinery strategy was focused on the separation of LS from the SSL using isopropanol and the use of the sugar-rich stream for the production of succinic acid via microbial fermentation (Fig. 1A). The second biorefinery process was based on the extraction of both LS and phenolic-rich extract from SSL prior to succinic acid production using the remaining detoxified solution. The LS were separated from the retentate stream obtained via nanofiltration using membranes with molecular weight cut-off (MWCO) of 500 Da and 800 Da, whereas the phenolic-rich extract was obtained via ethyl acetate extraction of the permeate stream (Fig. 1B). Fig. 1 presents the material balances for each biorefinery concept using as basis the treatment of 100 g of SSL.

2.2. Nanofiltration of SSL

Nanofiltration of SSL was carried out by AVECOM NV (Belgium) using a vibratory shear-enhanced processing filtration unit (V-SEP, New Logic Research, Emeryville, CA). A high shear at the surface of the filter membrane is achieved in a V-SEP filter via oscillatory vibration leading to significant improvement of the filter's resistance to fouling. This provides high throughputs and minimal reject volumes. The membranes used in the V-SEP filter for nanofiltration of SSL had MWCO of 500 Da (thin film non-polyamide, NF-500) and 800 Da (polyethersulfone, NF-PES-10). Before filtration, the SSL was diluted 7 times. The surface area of each membrane was 0.045 m^2. Each filtration run started with 28–35 L of the 7 times diluted SSL.

2.2.1. Membrane filtration with 800 Da MWCO

The filtration unit was initially operated for 2 h in a closed circuit mode. The filtrate was returned to the influent vessel in order to avoid up-concentration of the influent. This preliminary membrane test was performed in order to determine the membrane flux under more or less steady-state conditions.

Around 28 L of 7 times diluted SSL were used in the nanofiltration run carried out with 800 Da MWCO membrane. During open circuit filtration mode, the flow rate of the concentrate remained close to 450 L/h throughout filtration. In order to obtain an acceptable flux, the temperature and the pressure were increased during the filtration in the range of 38–54 °C and 10.5–27.8 bar, respectively. The flux through the membrane was gradually decreased from 34.7 L/(m^2 h) to 14.7 L/(m^2 h) during filtration. The permeate obtained was analyzed for sugars, LS and phenolic compounds and the corresponding values were 27.5 g/L, 20 g/L and 1.8 g/L.

2.2.2. Membrane filtration with 500 Da MWCO

As in the previous case, the filtration unit was initially operated for 1 h in a closed circuit mode. Then, an open circuit filtration mode followed in order to collect the permeate. Around 32 L of 7 times diluted SSL were used in the nanofiltration run carried out with 500 Da MWCO membrane. The flow rate of the concentrate remained close to 680 L/h throughout filtration. The temperature and pressure during filtration were increased in the range of 54–57 °C and 20.8–31 bar, respectively. The flux of the permeate through the membrane was decreased from 44 L/(m^2 h) to 10.7 L/(m^2 h) during filtration. The permeate obtained was analyzed for sugars, LS and phenolic compounds and the corresponding values were 22 g/L, 10 g/L and 1.6 g/L.

2.3. Extraction of phenolic compounds and lignosulphonates from SSL

Liquid–liquid extraction was employed to the nanofiltrated SSL using ethyl-acetate as solvent. The extraction was carried out at a pH value of 2.2 and a SSL-to-solvent ratio of 3.75 (v/v), using 100 mL of SSL in each extraction, as described by Alexandri et al. (2016). The adjustment of the pH was carried out using a HCl solution of 5 M. The solvent was removed via vacuum evaporation and reused. The crude extract was then re-dissolved in methanol and stored in −20 °C for further analysis.