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Possibility of L-(+)-lactic acid fermentation using malting, brewing, and oil production by-products

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ABSTRACT

Industrial by-products such as brewer's spent grain (BSG) hydrolysate, malt rootlets extract (MRE) and soybean meal extract (SME) were used for L-(+) lactic acid (LA) production by a pure *L. rhamnosus* ATCC 7469 strain. The effect of the addition of MRE (10–50%) or SME (10–50%) in BSG hydrolysate on batch and fed-batch LA fermentation was evaluated. The addition of MRE and SME increased the concentration of free amino nitrogen (FAN) and essential minerals (Fe, Mg, Mn, and Zn), which had a positive effect on the fermentation. Also, the MRE addition significantly lowered C/N ration to a more favorable level for the efficient LA fermentation. In batch fermentation, the highest LA concentration (25.73 g/L), yield (86.31%), and volumetric productivity (0.95 g/L h^{-1}), were obtained with the addition of 50% MRE. Further increase in LA concentration to 58.01 g/L, yield to 88.54%, and volumetric productivity to 1.19 g/L h^{-1} was achieved in fed-batch fermentation with addition of 50% MRE. A high optical purity of LA with 99.7% of L-(+)-isomer was obtained on the substrate based on industrial by-products. In addition, solid remains after BSG hydrolysis and MRE and SME preparation, together with the biomass of *L. rhamnosus* separated after the fermentation could be a good base for feed preparation.

1. Introduction

Lactic acid (LA) is the most important hydrocarboxylic acid with an asymmetrical carbon atom, widely distributed in nature, and used in food, pharmaceutical, textile, leather, and chemical industries (Wang, Chen et al. 2016a). Lactic acid has two optical isomers, L-(+)-LA and D-(−)-LA, which can be produced via either chemical synthesis or microbial fermentation. Chemical synthesis of lactic acid yields a racemic mixture of the two isomers, while, LA fermentation using an appropriate microorganism can produce optically pure L-(+)- or D-(−)-LA from renewable raw material (Gao et al., 2011; Mori et al., 2016). Presently, over 90% of LA is produced globally via fermentation, which is beneficial path due to environmental issue.

Lactic acid bacteria (LAB) are Gram-positive, facultative anaerobic bacteria that produce LA as the major fermentation product (Lew et al., 2013). Since LAB have limited potential to biosynthesize amino acids, nucleotides, and vitamins, supplementation of these nutrients is necessary for optimal growth. These complex nutritional requirements (especially in terms of carbon and nitrogen sources) increase significantly the costs of fermentation medium preparation (Okano et al., 2010). Usage of expensive refined sugars (mainly produced from crops that are used in human nutrition) in LA production is economically unfavorable and extremely limited due to increasing human population (Sauer et al., 2008). Lignocellulosic and starch materials, such as forestry and agricultural residues and food industry by-products, could be used as alternative feedstock for LA production without affecting the food supply (Chen et al., 2018). The valorization of lignocellulosic agrowastes as well as food industry by-products for production of LA will solve their disposal problem, environmental issues and eliminate dependence on petrochemical compounds. Their renewability, abundance, high polysaccharide content and non-competitiveness with food sources potentiate them as promising feedstocks for LA production (Grewal and Khare, 2018). Waste or by-products from agricultural and food industry such as brewer's spent grain (BSG), malt rootlets (MR) and soybean meal (SM) are cheap, nutritionally rich and abundant substrates that could be used for a sustainable LA production.

Brewer's spent grain (BSG) represents about 85% of the total by-products from brewing process and is available at low price during

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the whole year (0.1 $/kg) (Mussatto, 2014). The main use of BSG is as feed for animals such as ruminants. It is rich in carbohydrates-arabinoxylan and cellulose, lignin and protein, and can be used in chemical, food, and energy industries (Reis et al., 2015).

Malt rootlets (MR) are abundant and cheap by-product (0.19 $/kg) of malt industry, and they are discarded or valorized as animal feed. Rootlets are rich in B vitamins, vitamin E, peptides, amino acids, fatty acids, carbohydrates, dietary fibre, polyphenols, minerals and have antioxidant potential (Briggs et al., 1981; Bonnely et al., 2000). Research shows that rootlets application can be an interesting option from technical, sensory, nutritional, and economic perspectives (Waters et al., 2013).

Soybean meal (SM), a low cost by-product (0.52 $/kg) of soybean oil extraction, with a large amount of protein (approximately 50% w/w), mostly glycinin and betaconglycinin, carbohydrate, dietary fiber, vitamins and minerals, is the main protein ingredient in feeds (Shen et al., 2016; Seo and Cho, 2016; Stefanello et al., 2016). Compared with other plant proteins, it has a relatively high content of available protein, relatively well-balanced amino acid profile, steady supply, and reasonable price (Wang, Zhou et al., 2016b).

One of the main goals of "green" research on biomass utilization is development of combined technologies for integrated waste and by-product biorefinery concept, that will use mixed and multiple feedstocks to produce various products in the form of food, feed, fuel, power, and heat along with value-added chemicals (Nizami et al., 2017). One of the bottle-necks in commercial LA production on renewable materials is the substrate price, availability during the year and achievable nutrient and LA yields (Ghaffar et al., 2014). LA production processes developed on single waste substrate are highly susceptible to these variations (Refstie et al., 2005; Zhang et al., 2015; Polyorach et al., 2016; Cejas et al., 2017; Mathias et al., 2017). For the industrial scale it is critical to evaluate possibilities to substitute feedstocks in the case of variations in the prices and availability. We studied compatibility of different agri-food by-products and possibility to replace or interchange them for sustainable LAF as well as impact of substrate on optical purity of LA produced and critical characteristics of renewable substrates for effective LAF.

In this study BSG, MR and SM were combined and valorized as cheap raw materials in a fermentative LA production by *L. rhamnosus*. The combined usage of these substrates (obtained with chemical free or minimal chemical procedures) is more favorable in terms of resourceful waste management and production cost efficiency. The main goal was to evaluate the effect of the addition of malt rootles extract (MRE) (10, 30, and 50%) or soybean meal extract (SME) (10, 30, and 50%) as a free amino nitrogen and mineral source in BSG hydrolysate on LA fermentation parameters such as produced LA concentration, its productivity and yield, utilization of reducing sugar and *L. rhamnosus* cell viability. Fed-batch fermentation of BSG hydrolysate with malt rootlets or soybean meal extract and glucose addition during the fermentation was also evaluated. The combined mixtures were characterized by C/N ratio and related to resulting fermentation parameters. Solid remains after hydrolysis or extraction and remains after the fermentation were valorized as possible animal feed in order to design a sustainable LA production.

2. Material and methods

2.1. Brewer's spent grain hydrolysate preparation for fermentation

Prior to the LA fermentation BSG hydrolysis was optimized by the authors. Enzyme dosage was according to the producer's recommendation but was later increased according to the results of our investigation. Each enzyme (Termamyl SC® (α-amylase, EC 3.2.1.1), SAN Super 240L® (mainly amyloglucosidase, EC 3.2.1.3, and α-amylases, EC 3.2.1.1), and Cellic® CTec2 (cellulase complex, EC 3.2.1.4) was investigated on its own. After this a combination of these three enzymes was applied according to the best conditions obtained for each enzyme.

BSG obtained in a lager beer production was dried at 40 °C for 12 h. Dried BSG was finely ground in a laboratory DLFU mill from Bühler-Miag (Braunschweig, Germany). For hydrolysate production 50 g of dry BSG were mixed with 300 mL of distilled water and pH value of the obtained mash was adjusted to 5.5 with the addition of 10% H_3PO_4, prior to the hydrolysis. BSG hydrolysis was performed using automated mashing water bath (Glasbläserei, Institut für Gärungs Gewerbe, Berlin, Germany) by sequential adding of the following enzymes: 0.3 mL Termamyl SC (1 h at 90 °C), 0.3 mL SAN Super 240 L (1 h at 55 °C), and 4.0 mL Cellic® CTec2 (12 h at 45 °C) at 180 rpm. All commercial enzymes used in BSG hydrolysis (Termamyl SC, SAN Super 240 L, and Cellic® CTec2) were kindly provided by Novozymes (A/S Bagsvaerd, Denmark). After enzymatic hydrolysis obtained BSG hydrolysate was cooled to 20 °C and centrifuged (4000 rpm, 20 min, centrifuge: BOECO model C-28A, Hamburg, Germany). Liquid hydrolysate was separated from solid hydrolysate and used in LA fermentations. Its pH was adjusted to 6.5 with the addition of 1 M NaOH. After this, liquid hydrolysate was sterilized at 121 °C for 15 min and used as a fermentation medium. Reducing sugar concentration in hydrolysate was 3.5%.

2.2. Malt rootlets extract (MRE) and soybean meal extracts (SME) preparation for fermentation

Malt rootlets (MR) used in the experiments were obtained in malt production. Soybean meal (SM) used in the experiments was commercial. Production of malt rootlets extract and soybean meal extract was optimized (time of the extraction 1 and 2 h; temperature of the extraction; 60, 70 or 80 °C). Based on free amino nitrogen (FAN) concentration achieved, the best conditions were chosen.

For production of malt rootlets extract (MRE) or soybean meal extract (SME), 25 g of MR or SM was mixed with 200 mL of distilled water and extraction was carried out for 2 h at 60 °C and 80 rpm using automated mashing water bath (Glasbläserei, Institut für Gärungs Gewerbe, Berlin, Germany) after which MR or SM mash was centrifuged (4000 r/min, (2563*g*) 20 min) and MRE or SME was separated from the solid residue.

In batch and fed-batch LA fermentations MRE and SME were sterilized at 121 °C for 15 min and added in BSG hydrolysate (10, 30, and 50% v/v) prior to the inoculation.

2.3. Microorganism

Lactobacillus rhamnosus ATCC 7469, a homofermentative LA strain, was obtained from American Type Culture Collection (ATCC, Rockville, USA). Stock cultures of *L. rhamnosus* was stored and activated as previously described (Pejin et al., 2015). Inoculum was prepared by taking 3 mL of the activated culture and transferring it to 60 mL of MRS broth. To reach a high LAB cell number the inoculum was incubated for 24 h at 37 °C.

2.4. LA fermentation

All LA fermentations were performed with shaking (150 rpm, Biosan shaking bath model ES-20, Biosan Ltd., Latvia). The fermentations were performed in 300 mL Erlenmayer flasks with 200 mL of BSG hydrolysate. The fermentation was initiated by the addition of inoculum (5% v/v) and conducted at 37 °C. The pH was