Usage of food industry by-products as raw materials in lactic acid fermentation

Jelena Pejin[1], Miloš Radosavljević[1], Milana Pribić[1], Sunčica Kocić-Tanackov[1], Dragana Mladenović[2], Aleksandra Djukić-Vuković[2], Ljiljana Mojović[2]

[1]University of Novi Sad, Faculty of Technology, 21 000 Novi Sad, Bulevar cara Lazara 1, Serbia
[2]University of Belgrade, Faculty of Technology and Metallurgy, 11 000 Belgrade, Karnegijeva 4, Serbia
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Presenting author e-mail: jpejin@uns.ac.rs

Introduction

Lactic acid (LA) is a natural organic acid with application in the food, leather, cosmetic, and pharmaceutical industries (Cui *et al* 2012). The economics of LA production by fermentation is dependent on many factors, of which the cost of raw materials is significant. Lignocellulosic biomass is a promising raw material for LA production due to its sustainability, availability, and low cost compared to refined sugars (Abdel-Rahman *et al* 2011).

Brewer's spent grain (BSG), major by-product of the brewing industry, contains high concentration of fiber and protein as well as appreciable concentration of lipids, minerals, polyphenols, and vitamins. Malt rootlets (MR) are abundant and cheap by-product of malt industry, rich in B vitamins, vitamin E, peptides, amino acids, fatty acids, carbohydrates, fiber, polyphenols, and minerals (Briggs *et al* 2004). Soybean meal (SM) is a low cost by-product of soybean oil extraction, with high concentration of protein, carbohydrate, fiber, vitamins, and minerals (Shen *et al* 2016; Seo and Cho, 2016). In this study BSG hydrolysate was used in L-(+)-LA fed-batch fermentation by *Lactobacillus rhamnosus* ATCC 7469. Soybean meal extract (SME) and malt rootlets extract (MRE) were produced using optimized extraction procedure to achieve high free amino nitrogen (FAN) concentration.

The aim of this study was to evaluate fed-batch L-(+)-LA fermentation of BSG hydrolysate with MRE or SME addition in BSG hydrolysate and during fermentation and its effect on fermentation parameters (L-(+)-LA concentration, its productivity and yield, and *L. rhamnosus* cell viability).

Material and methods

BSG obtained in a lager beer production was dried at 40ºC for 12h. Dried BSG was finely ground in a laboratory DLFU mill from Bühler-Miag (Braunschwieg, Germany). Prior to the LA fermentation BSG hydrolysis (with the addition of commercial enzymes: Termamyl SC®, SAN Super 240L®, and Cellic® CTec2) was optimized. After enzymatic hydrolysis obtained BSG hydrolysate was centrifuged (4000 rpm, 20 min). Liquid hydrolysate was separated from solid hydrolysate and used in L-(+)-LA fermentations. Its pH was adjusted to 6.5 with the addition of 1M NaOH. After this, liquid hydrolysate was sterilized at 121°C for 15 min and used as a fermentation medium. Malt rootlets (MR) used in the experiments was obtained in malt production. Soybean meal (SM) used in the experiments was commercial. Production of malt rootlets extract and soybean meal extract was optimized and based on the highest FAN concentration achieved. *L. rhamnosus* ATCC 7469, a homofermentative LA strain, was obtained from American Type Culture Collection (ATCC, Rockville, USA). MRE and SME were added in BSG hydrolysate (10, 30, and 50% v/v) prior to the inoculation. All L-(+)-LA fermentations were performed with shaking (150 rpm). Fermentation was initiated by the addition of inoculum (5% v/v) and conducted at 37°C. The pH was maintained at 6.20 by the addition of a sterile 30% (w/v) NaOH solution in 4 hour intervals (Pejin *et al* 2017). Correction of reducing sugar concentration to the initial concentration (3.5%) was done with the addition of glucose and MRE or SME every 24 hours. Reducing sugar concentration, calculated as glucose, was determined by 3,5-dinitrosalicylic acid method (Miller 1959). L-(+)-LA concentration was determined by enzymatic method (L-(+)-LA assay, Megazyme, Wicklow, Ireland). FAN concentration in BSG hydrolysate, MRE, and SME was determined by ninhydrin method (MEBAK 2013). A number of viable *L. rhamnosus* cell was determined using a pour-plating method. Total viable cell number was expressed as log CFU mL^{-1}. The experiments were done in triplicates. All values are expressed as means ± standard deviation. Mean values of LA concentration, yield, and volumetric productivity, and *L. rhamnosus* cell viability were compared by the analysis of variance (one-way ANOVA) followed by Duncan test for mean differences testing. Differences were considered significant at $p<0.05$.

Results and discussion

FAN concentration significantly increased with the addition of MRE (by 113.96-364.31%) or SME (by 3.32-13.80%). The increase in L-(+)-LA concentration was in correlation with an increase in MRE or SME content, respecting FAN concentration in BSG hydrolysate. Significantly higher FAN concentration was achieved with the addition of MRE than SME.

In all fermentations high cell viability was achieved (9.4–9.5 log CFU mL^{-1}) (Figs. 1 and 2). MRE addition significantly increased L-(+)-LA concentration (by 18.92-47.31%) (Fig. 1). Also, SME addition increased L-(+)-LA concentration (by 1.61-4.21%) (Fig. 2). With an increase in MRE (by 0.74-3.73%) and SME (by 0.50-1.60%) content in BSG hydrolysate L-(+)-LA yield increased. Volumetric productivity was also increased with MRE (by 23.18-53.64%) and SME (by 0.76-3.08%) addition. The highest L-(+)-LA yield, volumetric productivity, and concentration of 88.54%, 1.19 g L^{-1} h^{-1}, and 60.89 g L^{-1}, respectively, were achieved in fermentation with the addition of 50% of MRE in BSG hydrolysate.

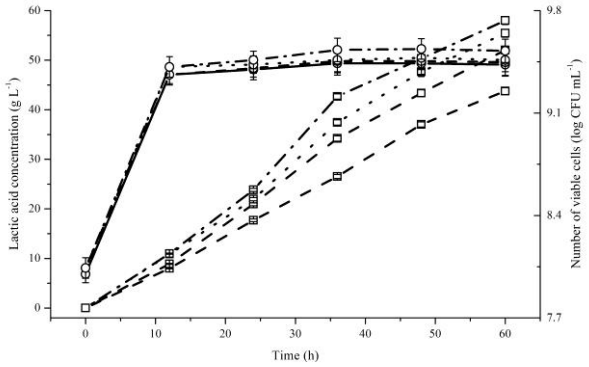

Figure 1. L-(+)-LA fermentation of BSG hydrolysate with MRE addition. Symbols: (□) L-(+)-lactic acid concentration; (○) *L. rhamnosus* cell viability; solid line – without MRE, dashed line – 10 % MRE; dotted line – 30% MRE; dash-dot line – 50% MRE.

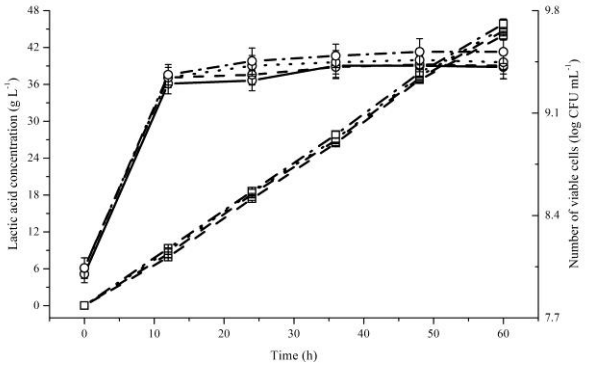

Figure 2. L-(+)-LA fermentation of BSG hydrolysate with SME addition. Symbols: (□) L-(+)-lactic acid concentration; (○) *L. rhamnosus* cell viability; solid line – without SME, dashed line – 10 % SME; dotted line – 30% SME; dash-dot line – 50% SME.

Conclusion

With MRE and SME addition high L-(+)-LA yield and volumetric productivity were achieved. The highest L-(+)-LA yield and volumetric productivity of 88.54% and 1.19 g/L h^{-1}, respectively, were achieved in fed-batch fermentation with the addition of 50% of MRE in BSG hydrolysate. This study has revealed that the combination of industrial by-products, BSG hydrolysate and MRE or SME can make a suitable fermentation media for L-(+)-LA fermentation. According to the obtained results, fed-batch fermentation could be used to increase L-(+)-LA fermentation efficiency. MRE and SME as nitrogen source and BSG hydrolysate (as a combined source of carbon and nitrogen) were good substrates for L-(+)-LA fermentation.

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