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Lactic acid fermentation of brewer's spent grain hydrolysate by *Lactobacillus rhamnosus* with yeast extract addition and pH control

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Lactic acid (LA) is a versatile chemical with a wide range of applications in food, pharmaceutical, cosmetic, textile and polymer industries. Brewer's spent grain (BSG) is the most abundant brewing by-product. In this study BSG hydrolysates were used for LA fermentation by *Lactobacillus rhamnosus* ATCC 7469. The aim of this study was to evaluate the effects of pH control during fermentation, reducing sugar content and yeast extract content in BSG hydrolysate on LA fermentation parameters. The pH control greatly increased reducing sugar utilization, L-(+)-LA content, yield and volumetric productivity. The highest L-(+)-LA yield and volumetric productivity were achieved with the reducing sugar content of 54 g/L. Yeast extract addition significantly increased reducing sugar utilization, L-(+)-LA content, *L. rhamnosus* cell viability, L-(+)-LA yield and volumetric productivity. The highest L-(+)-LA content (39.38 g/L), *L. rhamnosus* cell viability (9.67 log CFU/mL), L-(+)-LA yield (91.29%) and volumetric productivity (1.69 g/L/h) were achieved with the reducing sugar content of 54 g/L and yeast extract content of 50 g/L.

Keywords: lactic acid; fermentation; brewer's spent grain

Introduction

Demand for lignocellulosic biomass continues to increase for production of high-value chemicals and materials from renewable resources *(1)*. Brewer's spent grain (BSG) is the most abundant brewing by-product amounting to ~85% of total by-products generated by the brewing industry *(2)*. The chemical composition of BSG varies according to barley variety, harvest time, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process. In general, BSG is considered as a lignocellulosic material rich in protein and fibre, which account for around 20 and 70% of its composition, respectively. BSG also contains starch and its degradation products, lipids, amino acids, vitamins and minerals *(3,4)*. Millions of tonnes of BSG are produced annually across the world and common applications are direct disposal in a landfill or use as an animal feed *(2)*. Its possible applications are in human nutrition, as a raw material in biotechnology, energy production, etc. *(5)*.

Traditionally lactic acid (LA) is used in the food industry as a preservative, acidulant, flavouring agent or pH buffer. In recent decades, new technologies for processing LA allow further fields of applications, such as the production of biodegradable polymers, 'green' solvents and oxygenated, fine and commodity chemicals *(6)*. LA is industrially produced through either chemical synthesis or microbial fermentation. The advantage of the fermentation method resides in the fact that an optically pure LA can be obtained by choosing a strain of lactic acid bacteria, whereas chemical synthesis always results in a mixture of L-(+)- and D-(−)-LA *(7)*. Pure isomers, L-(+)- and D-(−)-LA, are more valuable than the racaemic DL form because each isomer has its own specific industrial application *(8)*.

Food and food-related applications currently account for ~85% of the demand for LA *(9)*. The LA market is forecast to reach 329,000 t in 2017 *(10)*. In order to deal with the increasing need and the resulting decreasing price, growing production costs and lessening availability of resources, current production procedures need to be optimized and new technologies are necessary *(11)*. Traditionally, LA is mostly produced by fermentation of starchy substrates like corn or potato but because of the competition of these substrates with food, lignocelluloses and wastes are currently being studied as a new and promising feedstock *(12)*.

The production of LA from lignocellulosic materials can be performed by sequential steps of chemical and/or mechanical processing (in order to make the cellulose more accessible to the enzymes), enzymatic saccharification (for obtaining solutions containing glucose as a main sugar), and finally, the hydrolysate fermentation by microorganisms, especially *Lactobacillus* species *(13)*.

The largest and most diverse genus of LA bacteria is *Lactobacillus*, which includes species with very different biochemical and physiological properties along with special resistance against an acidic environment *(14)*. Most LA bacteria require a wide range of growth factors including amino acids, specific minerals, vitamins, fatty acids, purines and pyrimidines for their growth and

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biological activity *(15)*. Thus, the substrate composition and nutritional requirements of the strain considerably affect the overall performance of the fermentation *(16)*. In many fermentation studies, yeast extract is considered to be an essential nutrient for lactobacilli for efficient LA production *(17)*.

Djukić-Vuković et al. *(18)* used *Lactobacillus rhamnosus* ATCC 7469, *Lactobacillus paracasei* ssp. *paracasei* NRRL B-4564, *Lactobacillus casei* ssp. *casei* NRRL B-441 and *Lactobacillus pentosus* NRRL-227 strains in LA fermentation of distillery stillage. The highest LA content, yield, volumetric productivity and number of viable cells were obtained by *L. rhamnosus*. In our previous study *(19)* LA production by *Lactobacillus fermentum* PL-1 and *L. rhamnosus* ATCC 7469 in BSG hydrolysate were investigated. *L. fermentum* produced similar L-(+)- and D-(−)-LA contents while *L. rhamnosus* produced mostly L-(+)-LA (95–98%) in all fermentations. In *L. fermentum* fermentations very low LA yields (30–44%) as well as low volumetric productivities were achieved. Therefore, *L. fermentum* proved not to be a good *Lactobacillus* strain in LA fermentation of BSG hydrolysate. In *L. rhamnosus* fermentations much higher LA yield (98%) and volumetric productivity (0.52 g/L/h) were reached. Owing to these results *L. rhamnosus* was chosen as a producing microorganism in this study.

Hydrolysates obtained from BSG *(13)* and other cellulosic materials such as wheat straw *(20)*, corn cobs *(21)*, wood *(22)*, cassava bagasse and sugarcane bagasse *(23,24)* required additional nutrients (yeast extract, de Man Rogosa Sharpe (MRS) medium, corn steep liquer, petone, different salts, etc.) for LA production by *Lactobacillus* strains.

In this study BSG hydrolysates were used for LA fermentation by *L. rhamnosus*. Firstly, the effect of pH control during fermentation of BSG hydrolysate on LA fermentation parameters was evaluated. Secondly, three different initial reducing sugar contents (27, 54 or 81 g/L) were evaluated and according to the obtained values of LA fermentation parameters an optimal reducing sugar content was selected. Finally, the effect of the addition of different yeast extract content in BSG hydrolysate on LA fermentation parameters was evaluated.

Materials and methods

Brewer's spent grain composition

BSG was monitored for the following quality parameters and the following methods were used for analysis: soluble extract, available residual extract and total residual extract (% dry matter) according to MEBAK *(25)*, protein content (% dry matter) by Kjeldahl method *(26)*, starch content after Ewers's polarimetric method (% dry matter) *(27)* and cellulose content (percentage dry matter) by Kirschner and Hoffer method. BSG before enzymatic hydrolysis was dried and analysed. After enzymatic hydrolysis liquid hydrolysate was separated from solid hydrolysate and used in LA fermentation. The solid residue after hydrolysis was dried and analysed. All BSG analyses were carried out in triplicate. Results were represented as means ± standard deviation.

Brewer's spent grain hydrolysate preparation

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 since the reducing sugar content increased. Each enzyme (Termamyl SC®, SAN Super 240 L®, and Celluclast 1.5 L®) 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 (Bühler-Miag, Braunschweig, Germany). For hydrolysate production 50 g of dry BSG were mixed with 300 mL of distilled water and the pH of the obtained mash was adjusted to 5.5 with the addition of H_3PO_4 (100 g/L), prior to hydrolysis. BSG hydrolysis was optimized during the preliminary studies and further performed using the optimized procedure. The hydrolysis was carried out using an automated mashing water bath (Glasbläserei, Institut für Gärungs Gewerbe, Berlin, Germany) by sequential addition of the following enzymes: 0.3 mL Termamyl SC® (α-amylase, EC 3.2.1.1; 1 h at 90°C), 0.3 mL SAN Super 240 L® (mainly amyloglucosidase, EC 3.2.1.3, and α-amylases, EC 3.2.1.1; 1 h at 55°C) and 5.0 mL Celluclast 1.5 L® (cellulase, EC 3.2.1.4; 10 h at 45°C) at 180 rpm. Prior to the addition of Celluclast 1.5 L® the pH was adjusted to 5.0 with the addition of H_3PO_4 (10%). All commercial enzymes used in BSG hydrolysis (Termamyl SC®, SAN Super 240 L®, and Celluclast 1.5 L®) were kindly provided by Novozymes (A/S Bagsvaerd, Denmark). Reducing sugar content in BSG hydrolysate obtained under these conditions was 27 g/L.

After enzymatic hydrolysis obtained BSG, the hydrolysate was cooled to 20°C and centrifuged (4000 rpm, 20 min; centrifuge was a BOECO model C-28A, Hamburg, Germany). Liquid hydrolysate was then 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, the liquid hydrolysate was sterilized at 121°C for 15 min and used as a fermentation medium.

Microorganisms

L. rhamnosus ATCC 7469, a homofermentative L-(+)-LA strain, was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Stock cultures of *L. rhamnosus* were stored at −20°C in 3 mL vials containing MRS medium (Fluka, USA) and 50% (*v*/v) glycerol as a cryoprotective agent.

L. rhamnosus cultures were activated after storage at −20°C: 0.2 mL of culture in MRS and glycerol was transferred to 7 mL of MRS broth and incubated for 48 h at 37°C. This procedure was repeated after 48 h. Inoculum was prepared by taking 3 mL of the activated culture and transferring it to 60 mL of MRS broth. To reach high LAB cell number the inoculum was incubated for 24 h at 37°C.

Lactic acid fermentation

In the first set of experiments the effect of pH control during LA fermentation was investigated. The pH was maintained at 6.2 (defined as optimal during the preliminary studies) by the addition of sterile NaOH (30%) in 4 h intervals.

In the second set of experiments the optimal initial reducing sugar content (27, 54, and 81 g/L) in BSG hydrolysate was investigated. Initial reducing sugar content in hydrolysate was set to 54 and 81 g/L by the addition of sterile 70% (*w*/*v*) glucose solution. After selection of the initial reducing sugar content the addition of yeast extract in BSG hydrolysate was investigated. The yeast extract content in BSG hydrolysate was set to 5, 10, 20, 30, 40 or 50 g/L (HiMedia Laboratories Ltd, Mumbai, India) prior to the sterilization. The fermentations with reducing sugar content of 54 or 81 g/L and yeast extract addition were performed with pH control.

All LA fermentations were performed as batch cultures with shaking (150 rpm, Biosan model ES-20, Biosan Ltd, Lithuania). 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.

Analytical methods

Reducing sugar content, calculated as glucose, was determined by 3,5-dinitrosalicylic acid method *(28)* using UV–vis spectrometer (UV-1800, Shimadzu, Kyoto, Japan). A calibration curve was set at 570 nm using standard glucose solutions. L-(+)-LA content was determined by enzymatic method [L-(+)-LA assay, Megazyme, Wicklow, Ireland]. Prior to the LA determination, proteins were removed from samples by precipitation according to the procedure prescribed in the L-(+)-LA assay (Megazyme, Wicklow, Ireland). The number of viable *L. rhamnosus* cells was determined using the pour-plating method. Microaerophilic conditions were maintained during incubation in Petri plates using a double MRS medium layer. Samples were incubated for 48 h at 37°C. Total viable cell number was expressed as log CFU/mL. All chemicals used in experiments were of analytical and microbiological grade.

Statistical analysis

The experiments were performed in triplicate. All values are expressed as means ± standard deviation. Mean values of treatments were compared by the analysis of variance (one-way ANOVA) followed by Duncan test for mean differences testing (SPSS Statistica 20, IBM Corporation, Armonk, New York, USA). Differences were considered significant at $p < 0.05$.

Results and discussion

The composition of BSG before and after enzymatic hydrolysis is presented in Table 1. BSG used in this study was produced in high-gravity wort production. During hydrolysis soluble and available residual extract content and cellulose content decreased while starch completely degraded. Protein content just slightly decreased because proteolytic enzymes were not used in the hydrolysis.

Table 1. Brewer's spent grain (BSG) composition before and after enzymatic hydrolysis[a]

Parameter	Before enzymatic hydrolysis	After enzymatic hydrolysis
Soluble extract, % dry matter	5.84 ± 0.27	0.23 ± 0.11
Available residual extract, % dry matter	4.47 ± 0.33	1.63 ± 0.14
Total residual extract, % dry matter	10.31 ± 0.37	1.86 ± 0.16
Proteins, % dry matter	26.48 ± 0.35	26.23 ± 0.34
Starch, % dry matter	4.10 ± 0.27	Not detected
Cellulose, % dry matter	15.15 ± 0.38	3.25 ± 0.09

[a]Values represent means ± standard deviation calculated from three parallel determinations.

Effect of pH control on lactic acid fermentation

Reducing sugar and L-(+)-LA content, *L. rhamnosus* cell viability and pH in LA fermentation of BSG hydrolysate without and with pH control are given in Fig. 1. L-(+)-LA yield and volumetric productivity in LA fermentation of BSG hydrolysate without and with pH control are presented in Table 2.

The pH control clearly affected the kinetics of reducing sugar utilization, with much slower reducing sugar utilization and conversion to L-(+)-LA in the fermentation without pH control (Fig. 1a). With pH control the reducing sugar utilization increased significantly (by 68.22%) compared with the fermentation without the pH control. The pH control increased L-(+)-LA content significantly, i.e. by 91.97% (Fig. 1b). *L. rhamnosus* cell viability increased during the fermentation without and with pH control and was similar in both fermentations (Fig. 1c). In the fermentation without pH control the pH decreased quickly (in 10 h of fermentation), which clearly affected the fermentation parameters (Fig. 1d). The pH control increased L-(+)-LA yield by 16.07%. Much lower volumetric productivity was achieved in fermentation without pH control. In both fermentations the highest volumetric productivity was achieved after 12 h (Table 2). According to the obtained results in further experiments the pH was controlled during all fermentations.

The pH is one of the main factors influencing LA production by fermentation process because the catalytic activity of the enzymes and the metabolic activity of the microorganisms depend on the extracellular pH *(13)*. Without pH control, the fermentation medium pH decreases with increasing LA production, resulting in the inhibition of cell growth and LA production *(29)*. The mechanism of LA inhibition of growth and metabolism in *L. rhamnosus* is a complex process and cannot be described as depending on only one type of LA/lactate equilibrium, nor can it be generalized for a wide range of extracellular pH values *(30)*. With pH control, a pH value closer to the optimal for LA acid bacteria cell metabolism (5.5–6.5) was achieved *(31)*. Idris and Suzana *(32)* reported the effect of initial pH from 4.5 to 8.5 on LA production during batch fermentation by *Lactobacillus delbrueckii* ATCC 9646. An initial pH of 6.5 caused the early induction of sugar conversion, maximum rate of sugar conversion and maximum LA content.

Effect of the reducing sugar content in BSG hydrolysate on LA fermentation

In further experiments an optimal initial reducing sugar content (27, 54 and 81 g/L) in BSG hydrolysate was investigated. Reducing sugar and L-(+)-LA content, *L. rhamnosus* cell viability and pH in LA fermentations of BSG hydrolysate with various initial reducing sugar contents are given in Fig. 2. L-(+)-LA yield and volumetric productivity in LA fermentations of BSG hydrolysate with various initial reducing sugar contents are presented in Table 3.

The highest sugar utilization was achieved in LA fermentation with reducing sugar content of 54 g/L (Fig. 2a). The highest L-(+)-LA content (21.29 g/L) was achieved with reducing sugar content of 81 g/L. However, high L-(+)-LA content (20.69 g/L) was also achieved with reducing sugar content of 54 g/L. Fermentation rate was higher in fermentation of hydrolysate with reducing sugar content of 54 g/L since the highest L-(+)-LA content was achieved after 36 h (Fig. 2b). Mussatto et al. *(13)* achieved lower LA content (12.76 g/L) in fermentation by *L. delbrueckii* UFV H2B20 on BSG hydrolysate with an initial glucose content of 50 g/L and with pH

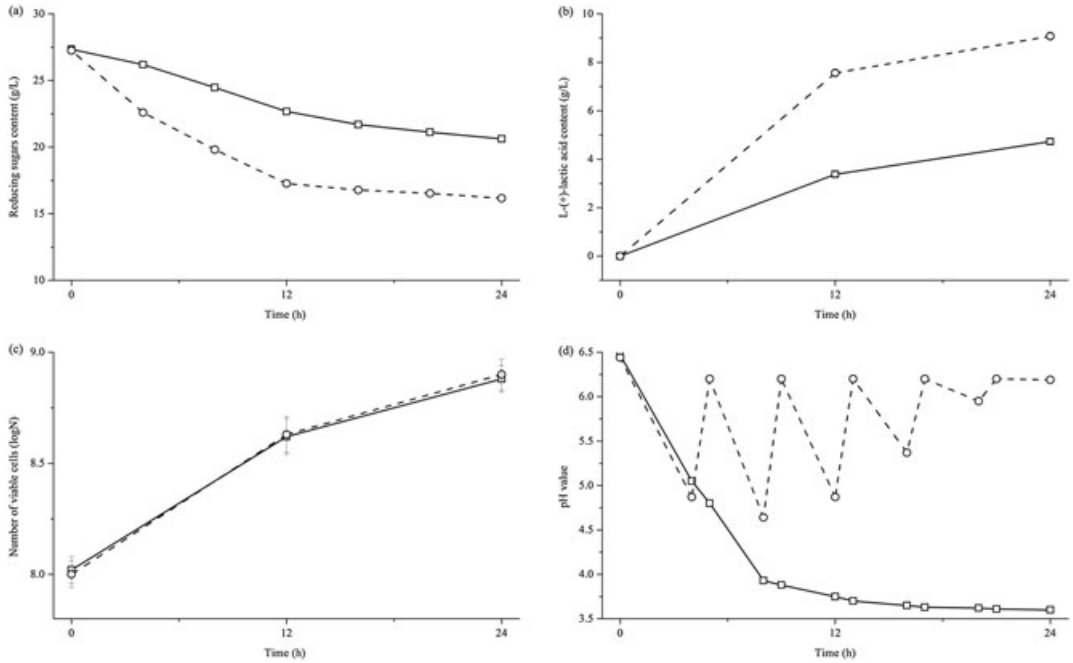

Figure 1. Lactic acid (LA) fermentation of brewer's spent grain (BSG) hydrolysate without and with pH control: (a) reducing sugar content; (b) L-(+)-LA content; (c) *Lactobacillus rhamnosus* cell viability; (d) pH. Symbols: (□) solid line – without pH control; (○), dashed line – with pH control.

Table 2. L-(+)-Lactic acid (LA) yield and volumetric productivity in BSG hydrolysate fermentation without and with pH control[a]

	L-(+)-LA yield (%)[b]	Volumetric productivity (g/L/h)[c]
Without pH control	70.47 ± 1.61[A]	0.28 ± 0.01[A]
With pH control	81.80 ± 0.85[B]	0.63 ± 0.01[B]

[a]Values represent means ± standard deviation calculated from three parallel experiments. Means written with the different capital letter in a column are significantly different ($p < 0.05$).
[b]L-(+)-LA yield was calculated at 24 h of the fermentation and expressed as grams of L-(+)-LA produced per gram of sugar consumed.
[c]Volumetric productivity was calculated at 12 h of fermentation.

control. *L. rhamnosus* cell viability (at the end of fermentation) in BSG hydrolysate fermentations was similar in fermentations with reducing sugar content of 54 and 81 g/L, while it was lower in the fermentation with lowest reducing sugar content of 27 g/L (Fig. 2c). According to the pH decrease LA fermentation was the most intense in the first 12 h (Fig. 2d). The highest L-(+)-LA yield and volumetric productivity were achieved with the reducing sugar content of 54 g/L (Table 3). Lower L-(+)-LA yield and volumetric productivity were obtained in fermentations with higher reducing sugar content (81 g/L). According to the obtained results in further experiments, the reducing sugar content was set to 54 g/L and the addition of different yeast extract contents was evaluated.

Similar results were obtained by Djukić-Vuković et al. *(33)*. These authors achieved the highest L-(+)-LA yield in fermentation of distillery stillage by *L. rhamnosus* ATCC 7469 with initial reducing sugar content of 55 g/L, and it declined with an increase in reducing sugar content to 85 g/L. They concluded that inferior reducing sugar conversion in fermentation with higher initial reducing sugar content could be due to an inhibitory effect of the produced L-(+)-LA. Hujanen et al. *(34)* observed a similar effect of an increase in L-(+)-LA content and a decline in LA yield when the initial sugar content increased from 80 to 120 g/L in LA fermentations with *L. casei* NRRL B-441 on semi-synthetic media containing different salts, glucose, yeast extract and/or malt sprout extract. Bai et al. *(35)* observed a progressive decline in volumetric productivity by increasing the initial reducing sugar content from 3 to 9% in LA fermentation of synthetic medium (glucose with the addition of salt and Tween 80) by *L. lactis* BME5-18 M.

Effect of yeast extract addition on LA fermentation

Reducing sugar and L-(+)-LA content, *L. rhamnosus* cell viability and pH in LA fermentation of BSG hydrolysate with various yeast extract content are given in Fig. 3. L-(+)-LA yield and volumetric productivity in LA fermentation of BSG hydrolysate with various yeast extract content are presented in Table 4.

Most *Lactobacillus* require an exogenous nitrogen source of amino acids or peptides to meet cell growth *(36)*. *L. rhamnosus* requires a complex nutrient composition for its growth because it lacks an enzyme to self-synthesize B vitamins and amino acids *(37)*.

Yeast extract addition increased reducing sugar utilization significantly [by 58.78% (5 g/L of yeast extract) to 71.46% (50 g/L of yeast extract)] compared with the fermentation without yeast extract addition (Fig. 3a). L-(+)-LA content increased significantly [from 68.39% (5 g/L of yeast extract) to 90.33% (50 g/L of yeast

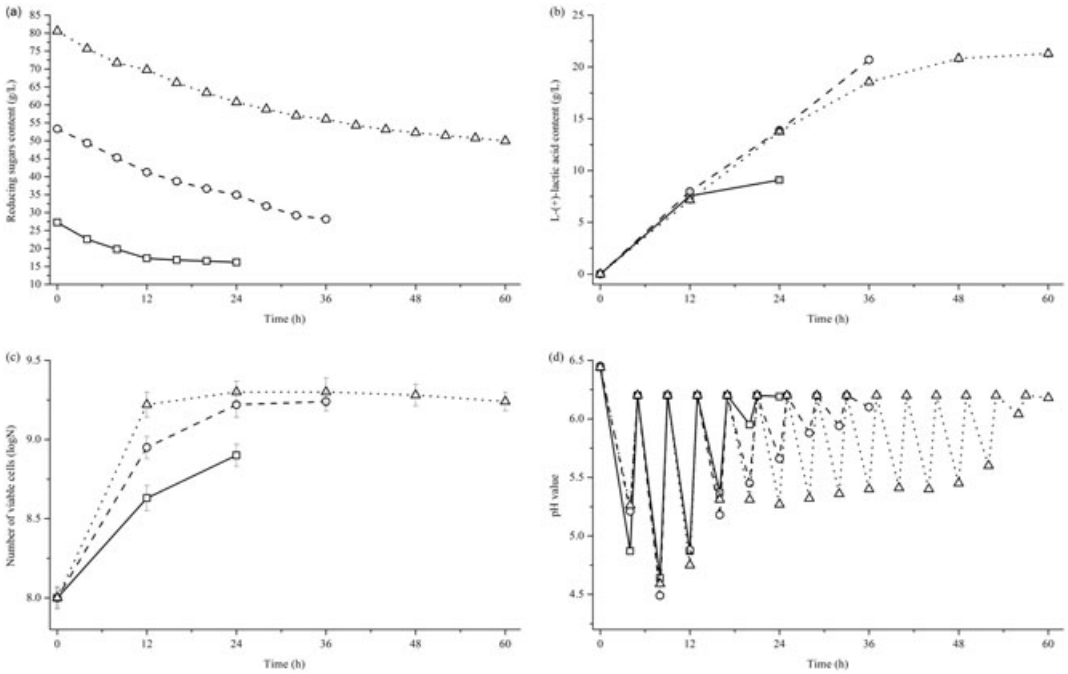

Figure 2. LA fermentation of BSG hydrolysate with various initial reducing sugar content: (a) reducing sugar content; (b) L-(+)-LA content; (c) *L. rhamnosus* cell viability; (d) pH. Symbols: (□), solid line – 27 g/L of reducing sugar; (○), dashed line – 54 g/L of reducing sugar; (Δ), dotted line – 81 g/L of reducing sugar.

Table 3. L-(+)-LA yield and volumetric productivity in BSG hydrolysate fermentations with various initial reducing sugar content[a]

Reducing sugar content (g/L)	L-(+)-LA yield (%)[b]	Volumetric productivity (g/L/h)[c]
27	81.80 ± 0.85[B]	0.63 ± 0.01[B]
54	82.25 ± 1.17[B]	0.67 ± 0.01[C]
81	69.55 ± 0.95[A]	0.60 ± 0.01[A]

[a]Values represent means ± standard deviation calculated from three parallel experiments. Means written with different capital letter in a column are significantly different ($p < 0.05$).
[b]L-(+)-LA yield was expressed as grams of L-(+)-LA produced per gram of sugar consumed (for initial reducing sugar content of 27, 54 and 81 g/L, it was calculated at 24, 36 and 60 h of fermentation, respectively).
[c]Volumetric productivity was calculated at 12 h of fermentation.

extract)] with yeast extract addition compared with the content in fermentation without the addition (Fig. 3b). Similar LA content was obtained by Mussatto et al. *(13)*. In fermentation of BSG hydrolysate with initial reducing sugar content of ~50 g/L, with the addition of MRS broth nutrients (containing 5 g/L of yeast extract, without glucose), and with pH control they achieved LA content of 35.54 g/L. *L. rhamnosus* cell viability was also significantly higher in fermentations with yeast extract addition [from 1.3% (5 g/L of yeast extract) to 4.6% (50 g/L of yeast extract) at the end of fermentation] than in fermentation without the addition (Fig. 3c). The addition of yeast extract affected pH decrease during fermentation. Lower pH values were obtained in fermentations with yeast extract addition (Fig. 3d). L-(+)-LA yield was high in all fermentations with yeast extract addition [87.22% (5 g/L of yeast extract) – 91.29% (50 g/L of yeast extract)]. Yeast extract addition increased L-(+)-LA yield significantly by 6.05% (5 g/L of yeast extract) to 11.00% (50 g/L of yeast extract) compared with the yield obtained in fermentation without the addition. Volumetric productivity was significantly higher when yeast extract was added compared with the fermentation without the addition, with the highest value of 1.69 g/L/h achieved with the addition of 50 g/L of yeast extract. The highest volumetric productivity in all fermentations with yeast extract addition was achieved after 12 h (Table 4). L-(+)-LA yield and volumetric productivity increased significantly with yeast extract content increase.

Li et al. *(38)* used glucose solution (100 g/L) with the addition of 15 g/L of yeast extract and different salts in LA fermentation by *L. rhamnosus* LA-04-1. L-(+)-LA yield of 85% was achieved, which was similar to the L-(+)-LA yield achieved in this study for 10 g/L of yeast extract (88.19%). Cui et al. *(39)* used synthetic glucose and xylose mixture (in a ratio 3:1 *w*/w with total initial sugar content of 20 g/L) and NaOH-pretreated corn stover (21.60 g/L of glucose and 6.89 g/L of xylose) with the addition of 5 g/L of yeast extract and different salts in LA fermentation by *L. rhamnosus*. These authors achieved an L-(+)-LA yield of 79% and volumetric productivity of 0.78 g/L/h (after 12 h of fermentation) on synthetic medium and L-(+)-LA yield of 59% and volumetric productivity of 0.49 g/L/h (after 36 h of fermentation) on NaOH-pretrated corn stover.

These results are lower than results obtained in this research for the same yeast extract content. However, the addition of higher concentrations of yeast extract is not economically feasible. In an economic analysis for LA fermentation, the largest contributor was found to be yeast extract, accounting for ~38% of medium cost. There is a need for investigation of the use of cheap renewable nitrogenous materials *(40)*.

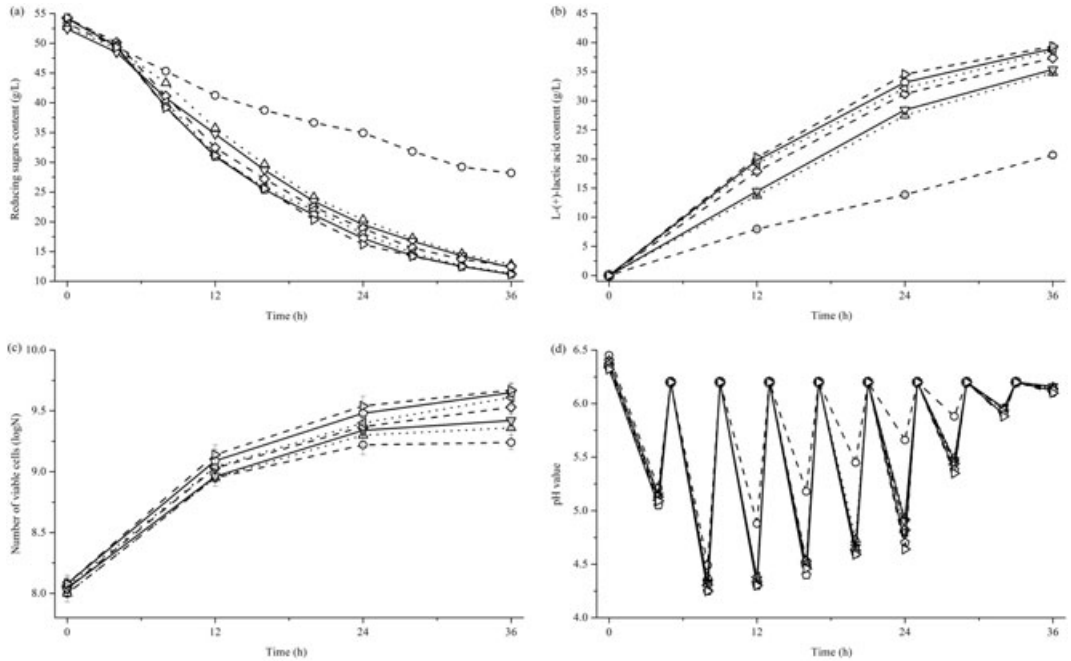

Figure 3. LA fermentation of brewer's spent grain (BSG) hydrolysate with various yeast extract content: (a) reducing sugar content; (b) L-(+)-LA content; (c) *L. rhamnosus* cell viability; (d) pH. Symbols: (○), dashed line – without yeast extract; (Δ), dotted line – 5 g/L of yeast extract; (▽), solid line – 10 g/L of yeast extract; (◊), dashed line – 20 g/L of yeast extract; (◁), dotted line – 30 g/L of yeast extract; (⌂), solid line – 40 g/L of yeast extract; (▷), dashed line – 50 g/L of yeast extract.

Table 4. L-(+)-LA yield and volumetric productivity in BSG hydrolysate fermentations with various yeast extract contents[a]

Yeast extract content in BSG hydrolysate (g/L)	L-(+)-LA yield (%)[b]	Volumetric productivity (g/L/h)[c]
0	82.25 ± 1.17^{A}	0.67 ± 0.01^{A}
5	87.22 ± 1.34^{B}	1.15 ± 0.02^{B}
10	88.19 ± 1.32^{BC}	1.21 ± 0.02^{C}
20	89.53 ± 1.59^{BCD}	1.49 ± 0.02^{D}
30	89.78 ± 1.65^{BCD}	1.60 ± 0.02^{E}
40	90.76 ± 1.65^{CD}	1.65 ± 0.02^{F}
50	91.29 ± 1.65^{D}	1.69 ± 0.02^{G}

[a]Values represent means ± standard deviation calculated from three parallel experiments. Means written with different capital letter in a column are significantly different ($p < 0.05$).
[b]L-(+)-LA yield was calculated at 36 h of fermentation and expressed as grams of L-(+)-LA produced per gram of sugar consumed.
[c]Volumetric productivity was calculated at 12 h of fermentation.

Conclusions

In this study the effects of pH control, initial reducing sugar content and yeast extract content were evaluated in LA fermentations of BSG hydrolysate. From the data obtained it was observed that pH control greatly increased reducing sugar utilization, L-(+)-LA content, yield and volumetric productivity. The highest L-(+)-LA yield and volumetric productivity were achieved with the initial reducing sugar content of 54 g/L. Yeast extract addition significantly increased reducing sugar utilization, L-(+)-LA content, *L. rhamnosus* cell viability, L-(+)-LA yield and volumetric productivity. The highest L-(+)-LA yield (91.29%) and volumetric productivity (1.69 g/L/h) were achieved with the initial reducing sugar content of 54 g/L and yeast extract content of 50 g/L. Since the addition of higher concentrations of yeast extract is not economically feasible future research must be conducted on screening of inexpensive renewable nitrogenous materials like industrial by-products and agricultural wastes/by-products (stillage obtained after bioethanol production, malt rootlet, soybean meal, etc.) as a substitute for yeast extract in LA fermentation.

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