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Evaluation of brewer's spent grain-derived lignocellulosic hydrolysate as a feedstock for amino acid production by submerged fermentation

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ABSTRACT

This study evaluated the potential of utilizing a lignocellulosic hydrolysate from brewer's spent grain (BSG) as a substrate for amino acid (AA) production by submerged fermentation. The main objective was to explore AA production from BSG hydrolysate using selected microorganisms. Initially, different microorganisms were screened for their growth on BSG hydrolysate, and selected microorganisms were further investigated for AA production by cultivation in shake flasks and bioreactor. From this screening, *Saccharomyces cerevisiae* and *Corynebacterium glutamicum* were selected. *C. glutamicum* produced alanine, proline, valine, and glycine in shake flasks and bioreactor. Highest alanine production (193.6±0.09 mg/L) was found in shake flasks after 30 h while production of proline (22.5±1.03 mg/L), valine (34.8±0.11 mg/L), and glycine (18.7±1.30 mg/L) was highest in bioreactor after 4 h (proline and valine) and 8 h (glycine). To enhance AA production by *C. glutamicum*, a fed-batch fermentation experiment was performed. Except for glycine, no AAs were produced during the fed-batch phase. *S. cerevisiae* produced alanine, proline, valine, and glutamic acid in shake flask but not in bioreactor. Highest production of alanine (11.8±1.25 mg/L), proline (11.8±1.06 mg/L), and valine (4.94±1.01 mg/L) was obtained after 50 h while glutamic acid production (66.2±0.49 mg/L) peaked after 60 h. This study demonstrates the production of several AAs from BSG by submerged fermentation; however, further optimization is needed to improve the productivity.

1. Introduction

Amino acids are compounds of immense industrial importance. They are used in several industrial applications including the manufacture of pharmaceuticals, human food, and animal feed [1,2]. A growing demand for amino acids has led to elevated global production from an estimated 10.8 million tons in 2022 to projected 14.3 million tons in 2028 [3]. This has fuelled a need for developing new and more sustainable technologies for amino acid production. Presently, amino acids are synthesized by three different routes: 1) chemical synthesis, 2) extraction from protein hydrolysates, and 3) microbial processes [4]. Microbial amino acid production occurs under submerged conditions by fermentation or enzymatic conversion. During enzymatic processes, medium sized peptides (30–40 amino acids) are hydrolyzed to form free amino acids. Through fermentation, sugars are converted to amino acids via intermediates of the central C-metabolism by using different microorganisms [5,6]. Microbial amino acid production offers several advantages over the chemical and extraction-based methods including mild chemical and physical conditions, low by-product formation, and the possibility of selective production of L-amino acids [6]. However, microbial production has shortcomings, specifically a high energy demand, the risk of contamination, and a large consumption of substrate, mainly glucose. Glucose is commonly produced from hydrolysis of starch which is also widely used in the food industry [7]. Due to an increasing demand for food, the use of starch-derived glucose in microbial processes is not sustainable and alternative substrates are needed. Recently, lignocellulosic biomasses have received considerable attention as renewable feedstock for microbial production due to their great abundance and high content of cellulose and hemicellulose which represent sources of fermentable sugars if broken down [8]. Moreover, current waste management practices of lignocellulosic biomasses are of environmental concern as they involve disposal in landfills and incineration. Therefore, implementation in fermentation processes, such as amino acid production, may alleviate the environmental burden posed by lignocellulosic biomasses while reducing the consumption of conventional fermentation substrates.

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Brewer's spent grain (BSG) is a plentiful, lignocellulosic by-product from beer production, which mainly consists of proteins (~20–30% of DM), lipids (~7–10% of DM), hemicellulose (30–35% of DM), cellulose (25–30% of DM) and lignin (~10–20 of DM) [9]. The main applications of BSG include feed supplementation for livestock (~70%), landfilling (~20%), and biogas production (~10%) [10]. However, due to its high and stable availability, low market price, and chemical composition, BSG constitutes a potential, yet untapped, resource for production of multiple compounds of industrial value [11]. Previous studies have demonstrated the use of BSG as a substrate for extraction of biopolymers – including protein [12,13], lignin [14,15], and hemicellulose [16] – and for production of various bio-based products, such as single cell protein (SCP) and enzymes (cellulose, lipases and amylases) [17–19], lactic acid [20], citric acid [21], and ethanol [22], using submerged or solid state fermentations. Even though extensive research has been carried out on the valorization of BSG, no previous studies have investigated the production of free amino acids from BSG. Given its chemical composition, free L-amino acids might be produced from BSG by two different approaches: 1) protein extraction followed by enzymatic/chemical hydrolysis of extracted proteins and 2) submerged fermentation using a lignocellulosic hydrolysate, yielded by hydrolysis of cellulose and/or hemicellulose, as fermentation substrate. In a sustainable context, fermentation-based amino acid production may be the most preferable strategy as it would enable value creation from the lignocellulosic fraction of BSG and, at the same time, increase the nutritional value of BSG by removing indigestible constituents such as cellulose and hemicellulose.

In this study, we examined the production of free amino acids from BSG-derived lignocellulosic sugars using submerged fermentation. Selected microorganisms were cultured in BSG hydrolysate-based media to evaluate the suitability of BSG-derived sugars as a fermentation substrate for amino acid production. Furthermore, we studied the impact of cultivation mode and growth phase on amino acid production. To our knowledge, this is the first study to demonstrate the potential of utilizing sugars sourced from the lignocellulosic fraction of BSG as a feedstock for production of free amino acids by submerged fermentation.

2. Materials and methods

2.1. Brewer's spent grain (BSG)

Brewer's spent grain was supplied by Carlsberg, Denmark. The raw BSG was autoclaved at 121 °C for 15 min and dried at 60 °C until the dry matter content was higher than 90%. Dried BSG was micronized at the Danish Technological Institute (Kolding) to obtain a size fraction of 125–250 μm. This fraction had the following proximate composition based on dry matter content: 20.2% protein, 20.0% cellulose, 28.7% hemicellulose, and 4.7% acid-insoluble lignin. Lignin, hemicellulose and cellulose were quantified by the Van Soest method [23] while the protein content was determined with the Kjeldahl method using a conversion factor of 6.25 [24].

2.2. Hydrolysis of brewer's spent grain

A BSG hydrolysate was produced by enzymatic hydrolysis of micronized BSG fraction using a modified version of the procedure outlined by Forssell et al. [25]. Briefly, the enzyme mixture Depol 686 L (Biocatalyst, UK) was added to a preheated suspension of BSG (10% w/v) in ammonium acetate buffer (50 mM, pH 5.0) to obtain a final enzyme activity of 106 U/mL. This mixture was incubated at 50 °C for 24 h with intermittent agitation. The solid and liquid fractions were separated by centrifugation at 3220 g and 21 °C for 25 min (5810 R Refrigerated Benchtop Centrifuge, Eppendorf, Germany). The liquid fraction was neutralized using a 10 M NaOH solution which resulted in formation of a white precipitate. This precipitate was removed by centrifugation at 3220 g and 4 °C for 25 min and discarded. For media with a pH below 7, pH of liquid fractions was adjusted using dilute H_2SO_4. Finally, the liquid fractions were filter sterilized (0.22 μm Sterivex-GV Pressure Filter, Merck Millipore).

2.3. Microorganisms

Four microorganisms were tested: *E. coli* ATCC 49161, *Lactobacillus plantarum* Lp 39, *Saccharomyces cerevisiae* DSMZ 70449, and *Corynebacterium glutamicum* DSMZ 1412. For each microorganism optimized cultivation conditions described in the literature were employed (media composition and pH, agitation speed and temperature). *C. glutamicum* was cultured adapting the conditions reported by Narayana et al. [26]. The cultivations of *E. coli*, *L. plantarum* and *S. cerevisiae* were carried out using the conditions defined by Blank et al. [27], Coelho et al. [28] and Van Hoek et al. [29], respectively.

2.4. Media composition

E. coli, *L. plantarum* and *S. cerevisiae* were cultivated in media with the following composition: KH_2PO_4, 0.052 g/L; $MgSO_4{\cdot}H_2O$, 0.036 g/L; Na_2HPO_4, 0.0164 g/L; $MnSO_4{\cdot}H_2O$, 0.00384 g/L; CH_3COONa, 0.0384 g/L; yeast extract, 1.0 g/L; BSG hydrolysate as the glucose source, 2.5 g/L. The medium pH was adjusted to 7.0±0.1 for *E. coli,* while a pH of 6.5±0.1 was used for *L. plantarum* and *S. cerevisiae*.

The medium used to culture *C. glutamicum* was prepared according to Narayana et al. [26] with few modifications: $MgSO_4{\cdot}H_2O$, 0.082 g/L; $(NH_4)_2SO_4$, 11.8 g/L; K_2HPO_4, 1.18 g/L; 3,4-dihydroxybenzoic acid, 0.35 mg/L; glucose added as BSG hydrolysate, 2.5 g/L. The medium pH was adjusted to 7.0±0.1.

The BSG hydrolysate and the 3,4-dihydroxybenzoic acid were sterilized by filtration while the rest of the media were autoclaved at 121 °C for 30 min

2.5. Inoculum preparation

The inoculum for the bioreactor cultivations was prepared as follows: 500 μL of 15% glycerol stock culture (OD_{600}: 7–8) was added to 100 mL sterile medium in a shake flask. The shake flask cultures were incubated in an orbital shaker (MaxQ 8000, Thermo Fisher Scientific, Marietta, USA) until their OD_{600} values reached 9–13 and 2.0–2.5 for *C. glutamicum* and *S. cerevisiae*, respectively. Then, these 100 mL shake flask cultures were used for inoculation of the bioreactor. Optimal speed and temperature were employed for each microorganism during inoculum production.

2.6. Shake flask cultivation

E. coli, *S. cerevisiae*, *L. plantarum*, and *C. glutamicum* were grown in 250 mL shake flasks containing 50 mL of sterile medium. Cultivations of *S. cerevisiae* and *L. plantarum* were performed at 160 rpm and 30 °C while *E. coli* was cultured at 250 rpm and 37 °C. Shake flask experiments with *C. glutamicum* were carried out at 130 rpm and 30 °C.

2.7. Bioreactor cultivations

Batch fermentations with *C. glutamicum* and *S. cerevisiae* were performed in a 3.5 L stirred bioreactor (Chemap, Switzerland) using an over pressure of 0.3 bar and constant supply of air (2.5 L/min) through the bottom sparger. The cultivation of both microorganisms was carried out at 30±0.1 °C. Dissolved oxygen (DO) was monitored with a DO probe (InPro6800, Mettler Toledo) and maintained at a minimum level of 40% air saturation by regulating agitation speed or airflow. The pH was measured using a pH probe (InPro3253, Mettler Toledo). For *C. glutamicum*, pH was maintained at 7.0±0.1 with 10 M NaOH and 2 M H_2SO_4. For *S. cerevisiae*, pH was kept at 5.0±0.1 with 1 M NaOH and

2 M H_2SO_4. Foaming was controlled by dropwise addition of an anti-foaming agent (Glanapon 2000, Sigma). Percentages of O_2 and CO_2 in the off-gas were measured continuously using a mass spectrometer (Prima Pro Process Mass Spectrometer, ThermoFisher) and the experimental data was acquired from Labview 6 (National Instruments, USA). During the batch fermentations with *S. cerevisiae* and *C. glutamicum*, 1.5 L medium was inoculated with 100 mL inoculum. The batch phase was terminated when off-gas CO_2 dropped.

In addition, a fed-batch fermentation was performed in bioreactor with *C. glutamicum*. Following a drop in off-gas CO_2, the fed-batch phase was initiated using an exponential feeding rate, Q(t), calculated by equation:

$$Q(t) = \frac{\mu_{\text{int}} \bullet M_{b,0} \bullet C_{x,0}}{C_{s,in} \bullet Y_{x/s}} \bullet \exp(\mu_{\text{int}} \bullet t) \quad (1)$$

Here, $C_{s,in}$ is the glucose concentration in the feed (g/L), $M_{b,0}$ is the initial weight of the fermentation broth (g), $C_{x,0}$ is the initial biomass of the fed-batch phase (g/L), and μ_{int} is the intended growth rate during the fed-batch phase (h^{-1}) which was estimated as 1/3 of the maximum growth rate, $\mu_{\max}$, obtained during the batch phase. $Y_{x/s}$ is the yield coefficient of biomass on substrate which was determined by Eq. 2:

$$Y_{x/s} = \frac{\Delta_{OD_{600}} \bullet F(= biomass \quad produced)}{\Delta C_{s,batch}(= substrate \quad consumed)} \quad (2)$$

where $\Delta_{OD_{600}}$ is the change in optical density during the batch phase, $\Delta C_{s,batch}$ is the substrate consumption during the batch phase, and F is a factor used to convert optical density into cell dry weight (CDW). A conversion factor of 0.3 g CDW/L/OD_{600} for *C. glutamicum* reported by Blombach et al. [30] was applied to calculate $Y_{x/s}$ in this study. The feed had same composition as the fermentation medium except for a higher glucose concentration (4 g/L).

2.8. Sampling procedure

Approximately 7 mL of sample was collected at regular intervals during the fermentation experiments. From each sample, 2 × 1 mL was used to determine optical density (OD_{600}) and the remaining was centrifuged at 1258 g and 4 °C for 15 min. After centrifugation, cell pellets were discarded while supernatants were filtered through a sterile filter (0.22 μm Sterivex-GV Pressure Filter unit, Merck Millipore) and used for amino acid and sugar quantification. The same procedure was deployed for the shake flask experiments except for the filtration step due to the small sampling volumes (1 mL).

2.9. Analytical procedures

2.9.1. Optical density

Biomass was measured as optical density at 600 nm (OD_{600}) using a spectrophotometer (Ultrospec 2100 pro spectrophotometer, Amersham Biosciences, Uppasala, Sweden).

2.9.2. Amino acids

The amino acid analysis was carried out in two steps, namely purification and quantification. In the first step, the samples were purified by Solid Phase Extraction (SPE) to eliminate interfering compounds. The SPE purification step was carried out using a strong cation exchange (SCX) cartridge (40–75 μm, 70 Å, 100 mg/1 mL, Hawach Scientific, China). Initially, the solid phase was equilibrated with 1 mL of 100% methanol and acidified with 1 mL of 1% (v/v) aqueous formic acid. Samples were diluted two-fold in 1% (v/v) aqueous formic acid and norvaline was added as internal standard (IS) as to obtain a final concentration of 100 μM in the diluted samples. Diluted samples (500 μL) were loaded onto the solid phase and washed twice with 500 μL of 1% (v/v) formic acid in methanol. Then, amino acids were eluted from the stationary phase with 2 × 250 μL 5% ammonium hydroxide in methanol. In the second step, concentrations of amino acids were determined according to the method described by Cohen [31]. The amino acids were converted into UV-active derivatives by reaction with the substrate 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate (AQC) using a commercial derivatization kit (AccQ•Tag Ultra Derivatization Kit, Waters, Milford, Massachusetts). Amino acid derivatives were then quantified using reversed-phase liquid chromatography with UV detection at 260 nm (Waters ACQUITY UltraPerformance liquid LC, ACCQ-TAG ULTRA C18, 2.1 ×100 mm, 1.7 μm column). Water (eluent A) and acetonitrile (eluent B) were used as eluents. A gradient of 0–60% eluent B over 10 min and a flowrate of 0.7 mL/s were applied to separate the amino acids.

2.9.3. Sugars, organic acids, and ethanol

Sugars (glucose, arabinose, and xylose), organic acids (lactate and acetate), and ethanol were quantified by HPLC analysis (Shimadzu Nexera XR) using refractive index detection (RID-20A). Compounds were separated by reversed-phase mode (Biorad Aminex HPX-87 H column) using the following conditions: 0.6 mL/min, 30 °C, and a 4 mM aqueous sulfuric acid eluent. Filtrated samples were prepared for injection (1 μL) by two-fold dilution (1:1) in 0.2% aqueous sodium azide. Quantification was performed by comparing samples with 10 g/L standard solutions of each compound.

2.10. Data analysis

All variables (OD_{600}, amino acids, and sugars) were determined in duplicates and results were reported as meansSD. All graphs were created in the software GraphPad Prism 9.3.1.

3. Results and discussion

3.1. Selection of microorganisms for amino acid production

The four microorganisms, listed in Table 1, were cultured in shake flasks to evaluate their ability to utilize BSG hydrolysate as a substrate for amino acid production. These microorganisms were chosen for this initial screening due to their previously reported ability to produce amino acids [5,32–34].

An initial screening of the microorganisms' growth was performed to select high biomass producers (OD_{600} >8) for further study of their amino acid production. Although *E. coli* and *L. plantarum* are well-known amino acid-producers [5,34] and have previously been used for production of different bioproducts, such as lactic acid and ethanol, from BSG [35,36], here they were not selected for further study of amino acid production due to their poor growth in BSG hydrolysate-based media. The growth of these microorganisms might have been limited by the drop in medium pH observed after cultivation. The pH dropped from 7.0 to 4.9 and from 6.5 to 3.8 during cultivation of *E. coli* and *L. plantarum*, respectively. This decrease may have been caused by the production of acetic acid by *E. coli* and lactic acid by *L. plantarum* although the presence of these metabolites was not confirmed by chemical analysis. Similarly, pH declined from 6.5 to 5.4 for *S. cerevisiae*; however, this decrease was not found to have any impact on growth since pH remained within the strain's previously reported optimum conditions (pH between

Table 1
Screening for growth and amino acid production.

Microorganism	Growth performance[a]	Main amino acids produced
E. coli	+	ND
L. plantarum	+	ND
S. cerevisiae	+ +	Ala, Pro, Glu, Val, Leu, Phe
C. glutamicum	+ +	Ala, Pro, Val, Gly

[a] Growth performance was based on optical density measured at 600 nm (OD_{600}). + : low growth (OD_{600} <8). + +: high growth (OD_{600} >8).

4.0 and 6.0) [37]. By contrast, pH had increased from 7.0 to 8.1 after 48 h cultivation of *C. glutamicum* which was also found to be within the optimum pH range (6.0–9.0) and therefore it did not affect the growth of the microorganism [38]. Based on these results, *C. glutamicum* and *S. cerevisiae* were selected for further study to explore their amino acid production.

3.2. Cultivation of Corynebacterium glutamicum and Saccharomyces cerevisiae

To develop a microbial process for transforming BSG-derived sugars into amino acids, *C. glutamicum* and *S. cerevisiae* were cultured in shake flasks and bioreactor. One shake flask experiment and two bioreactor cultivations were carried out for both microorganisms. The term amino acid profile refers to the concentration of free amino acids present in the fermentation broth at any given time.

Based on the changes in amino acid concentrations during growth, their consumption or production was determined.

3.2.1. Shake flask cultivations

Shake flask experiments were performed with the objectives of optimizing the media and investigating biomass growth and amino acid formation.

The shake flask experiment with *S. cerevisiae* was performed using BSG hydrolysate as C-source and yeast extract as N-source. Yeast extract also contains carbohydrates (~10% of dry matter) which could be utilized as C-source. To examine whether the BSG hydrolysate comprised the primary C-source for *S. cerevisiae*, media with high (1 g/L) and low (0.5 g/L) concentrations of yeast extract were tested. Reducing the concentration of yeast extract from 1 g/L to 0.5 g/L led to a slight decrease in the growth performance as OD_{600} dropped from ~8 to ~6 after 20 h (data not shown). This suggests that the carbohydrates in yeast extract supported growth of *S. cerevisiae*; however, yeast extract was not the main C-source. Furthermore, it is important to note that the observed decrease in OD_{600} could also be due to limitations of the N-source.

The growth medium for *C. glutamicum* did not contain yeast extract but ammonium sulfate as N-source. Chemical analysis revealed the presence of several free amino acids in this medium which may result from solubilization of amino acids during the enzymatic hydrolysis of BSG or simultaneous protein hydrolysis. However, low proteolytic activity has been reported for the Depol 686 L mixture and therefore the degree of protein hydrolysis was expected to be insignificant [25]. The effect of N-source on *C. glutamicum* growth and amino acid formation was investigated by using media with low (12 g/L) and high (20 g/L) concentrations of ammonium sulfate referred to as M_{Low} and M_{High}, respectively (Fig. S1). The growth curves obtained from cultivation of *C. glutamicum* in M_{Low} and M_{High} followed the same trend, although the bacterium appeared to grow to a slightly higher biomass when cultured in the M_{High} medium (Fig. S1A). On the other hand, *C. glutamicum* had highest production of alanine (Ala) in M_{Low} medium (~194 mg/L) compared to M_{High} medium (~135 mg/L) (Fig. S1B). As a result, M_{Low} medium was chosen for all subsequent cultivations of *C. glutamicum*.

A comparison of the growth data obtained for each microorganism reveals that *C. glutamicum* grew faster than *S. cerevisiae* on BSG hydrolysate (Fig. 1). For *C. glutamicum* an unusual growth pattern was observed during the stationary phase where OD_{600} first declined rapidly (~13 h) and then increased (~24 h) (Fig. 1A). The underlying cause of this observation is unclear; yet, it may be attributed to changes in the pH and DO as these parameters were not regulated during the shake flask experiments. Alternatively, it could be explained by an outlying data point; though, this scenario is unlikely because the same trend was obtained from cultivations in M_{Low} and M_{High} media. For *S. cerevisiae*, the shape of the growth curve suggested that its growth is sustained by sequential utilization of different substrates present in the BSG hydrolysate-based media during incubation (Fig. 1B). This phenomenon, also known as diauxic growth, is well-documented for *S. cerevisiae* in the literature. Collectively, previous studies have established that *S. cerevisiae* preferentially ferments sugars during the initial growth phase to produce different nonfermentable compounds, such as ethanol and acetic acid, which serve as substrates after sugar depletion [39–41]. For the shake flask cultivations reported here, the substrate utilization pattern in *S. cerevisiae* was not investigated further. Moreover, the differences in the growth profiles observed between *C. glutamicum* and *S. cerevisiae* might be related to the type and concentration of N-source; nevertheless, these aspects were not explored in the present study.

Different amino acid profiles were obtained from shake flask cultivations of *C. glutamicum* and *S. cerevisiae* (Fig. 2). It is important to be aware that BSG is a source of various protein-bound amino acids which may be solubilized during enzymatic hydrolysis of cellulose and hemicellulose. As a result, the BSG hydrolysate-based media may contain free amino acids that cannot be ascribed to microbial production, and which will lead to overestimation of amino acid production if not accounted for. To avoid this, concentrations of amino acids were determined in the growth media and used as a reference point for calculating the microbial production of free amino acids. *C. glutamicum* showed highest increase in Ala, valine (Val), and proline (Pro) (Fig. 2A). The maximum concentrations achieved for Ala, Val and Pro, after 30 h of cultivation, were

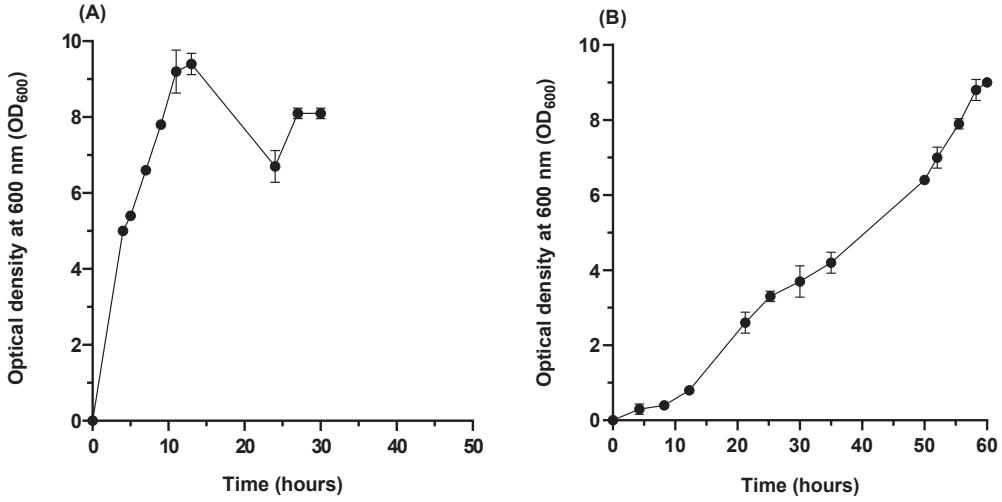

Fig. 1. Growth curves obtained from shake flask cultivation with Corynebacterium glutamicum (A) and Saccharomyces cerevisiae (B). Data is reported as the meanSD of two technical replicates.

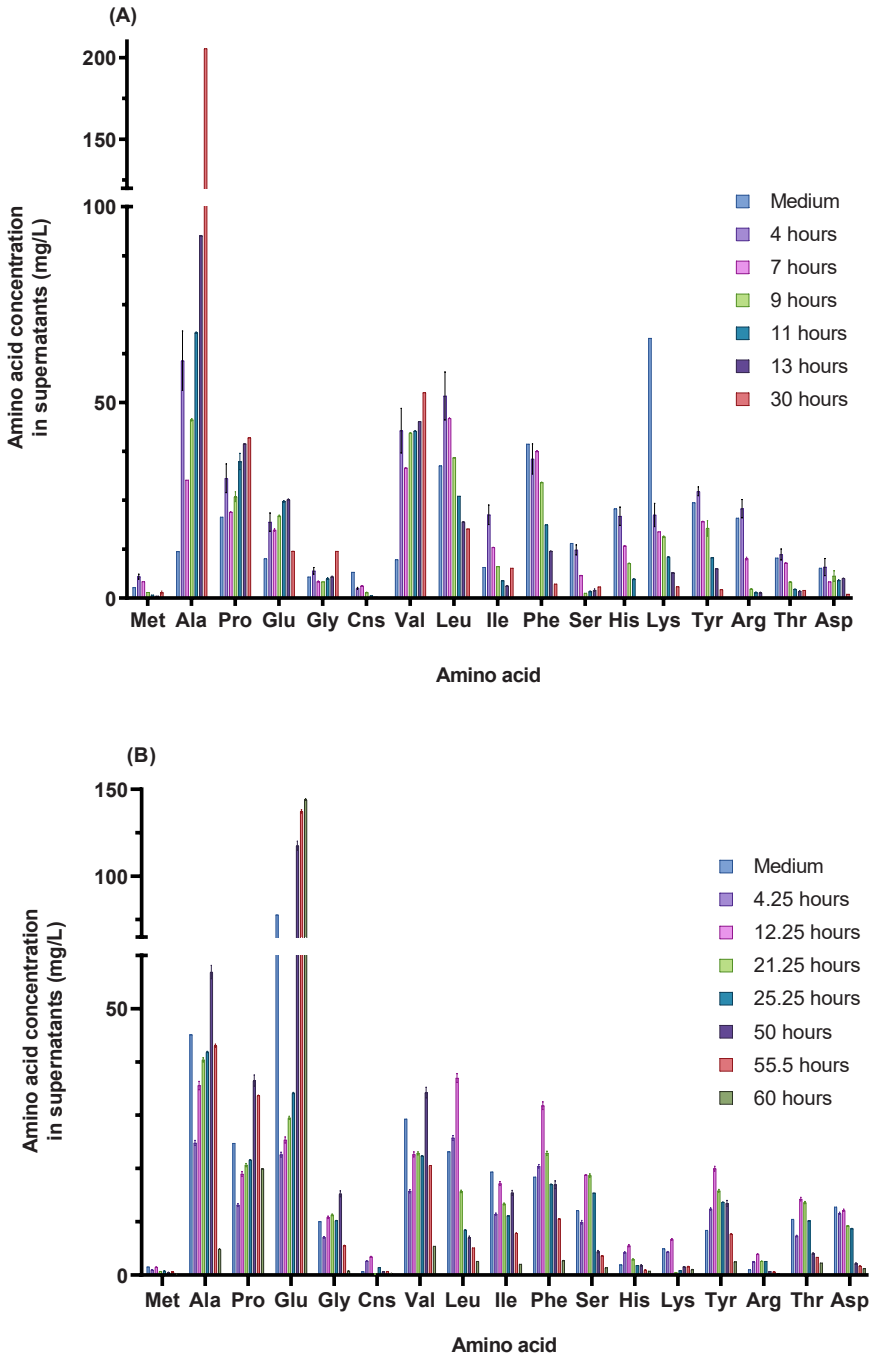

Fig. 2. Trends in amino acid concentrations (mg/L) during shake flask cultivations with Corynebacterium glutamicum (A) and Saccharomyces cerevisiae (B). Data is reported as the meanSD of two technical replicates.

205.5, 52.3, and 41.0 mg/L. This corresponds to a 17, 5, and 2 fold increase compared to the initial medium concentrations. For *S. cerevisiae*, several amino acids were produced with maximum yield observed at different times (Fig. 2B). These amino acids include Ala (45.1 mg/L to 56.9 mg/L), Pro (24.7–36.5 mg/L), glutamic acid (Glu) (77.8–144.1 mg/L), leucine (Leu) (23.2–37.0 mg/L), and phenylalanine (Phe) (18.4–31.8 mg/L). All amino acids were produced in small quantities except for Glu that nearly doubled during a growth period of 60 h.

3.2.2. Bioreactor cultivations

To examine the effect of controlled conditions (pH, aeration, agitation, etc.) on growth and amino acid production, fermentations were conducted with *S. cerevisiae* and *C. glutamicum* in a bioreactor.

3.2.2.1. Batch fermentations. Batch fermentations were performed in a 3.5 L Chemap bioreactor with media volume of 1.5 L using *C. glutamicum* and *S. cerevisiae*. To verify the reproducibility, duplicate batch fermentations were done for each microorganism, and trends in amino acid and growth profiles were studied (Fig. 3 and Fig. 4).

The growth curves and substrate profiles achieved for each microorganism are compared in Fig. 3. For *C. glutamicum*, the stationary growth phase was observed after 7–8 h with a maximum OD_{600}~10 (Figs. 3A and 3B). No decrease in OD_{600} was detected during the stationary phase as seen in the shake flask experiment (Fig. 1A). This finding supports our hypothesis that the decrease in OD_{600} observed

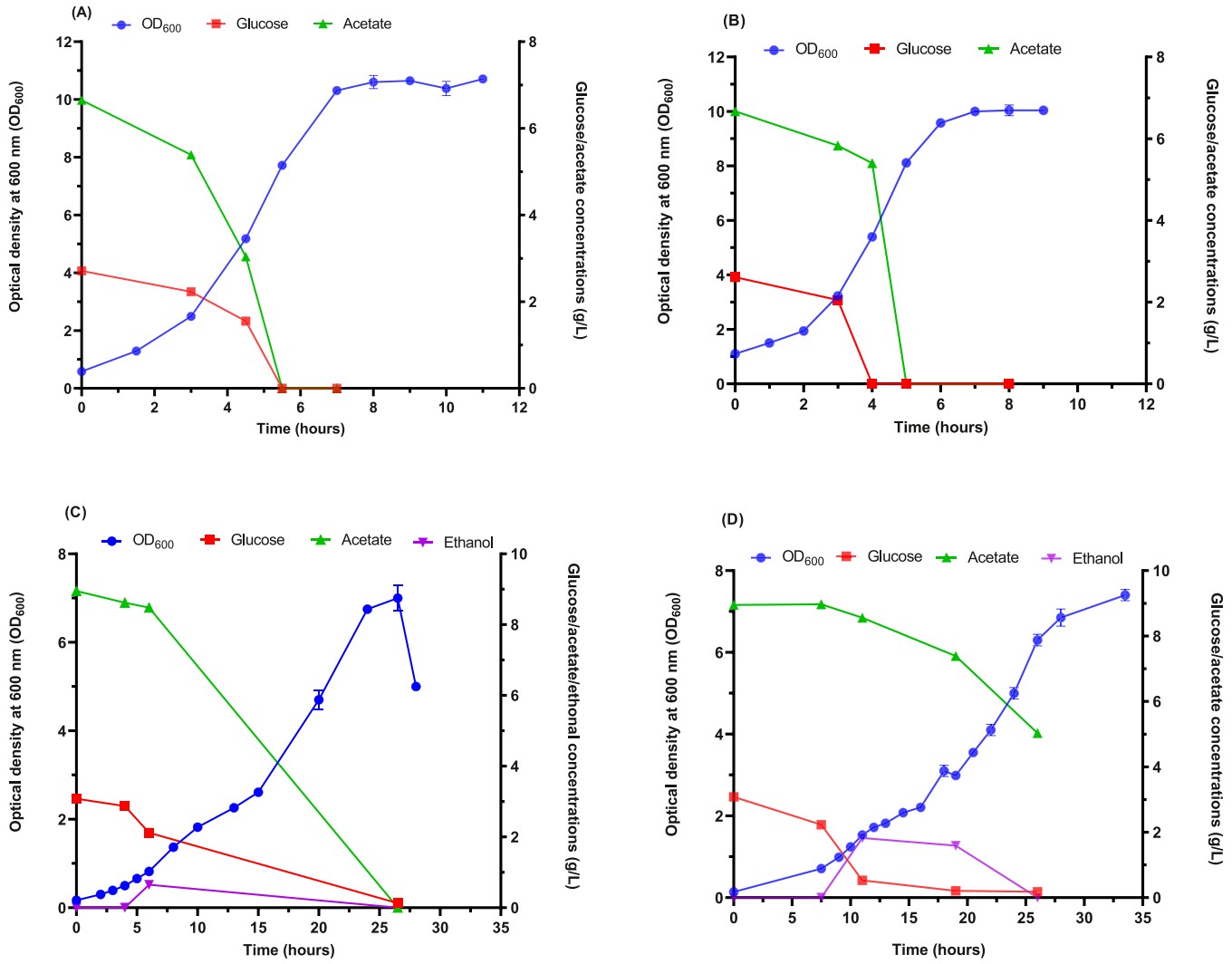

Fig. 3. Substrate (glucose and acetate) and product (biomass and ethanol) profiles during two repeated batch fermentations in bioreactor with Corynebacterium glutamicum (A, B) and Saccharomyces cerevisiae (C, D). Data is reported as meanSD of two technical replicates. Ethanol concentrations are only displayed for S. cerevisiae as C. glutamicum neither produced nor consumed ethanol during batch fermentation.

during the shake flask experiment resulted from fluctuations in pH and/or DO. The maximum OD_{600} values obtained for *C. glutamicum* in bioreactor and shake flask fermentations were similar (OD_{600}: 9–10) while the growth period was shorter in bioreactor (7–8 h) compared to that in shake flask (30 h). *S. cerevisiae* reached stationary growth phase after 26–28 h, much faster than in the shake flask experiment (60 h). Comparable OD_{600} values (OD_{600}: 7–9) were achieved in both cultivation modes (Figs. 3C and 3D).

Chemical analysis showed that the BSG hydrolysate-based media contained different concentrations of a variety of sugars and organic acids such as glucose (2.5–3.0 g/L), arabinose (1.5–2.0 g/L), xylose (2.6–3.5 g/L), acetate (6.7–8.9 g/L), and lactate (1.0–1.3 g/L). Glucose originated from the hydrolysis of cellulose, and to a lesser extent β-glucans, found in BSG. On the other hand, arabinose, xylose, and acetate were major products from hydrolysis of arabinoxylan, the most predominant hemicellulose in BSG [42]. Furthermore, the buffer solution (ammonium acetate) used for BSG hydrolysis also contributed to the high media concentrations of acetate. The results presented in Fig. 3 indicated that both microorganisms utilized glucose and acetate as major substrates while neither of them consumed xylose and arabinose (Fig. S2). For both microorganisms, lactate concentrations remained stable during the exponential phase but was depleted by the end of the cultivations. A possible explanation for this could be that lactate was consumed after the depletion of glucose and acetate. Furthermore, the substrate curves implied that *C. glutamicum* and. *S. cerevisiae* utilized glucose and acetate by two distinct mechanisms. For *C. glutamicum*, overlapping curves indicated simultaneous consumption of glucose and acetate (Figs. 3A and 3B). This result was consistent with findings from previous studies which demonstrated the capability of *C. glutamicum* to co-metabolize glucose with other substrates such as acetate, lactate, and various sugars [43]. For *S. cerevisiae*, consumption of glucose was observed within the first 10 h after which acetate concentrations began to decrease (Fig. 3D). In addition, increasing ethanol concentrations were observed during the early growth phase (~6 h) indicating that ethanol was produced by *S. cerevisiae* in BSG hydrolysate-based medium (Fig. 3D). This result can be attributed to the Crabtree effect; a metabolic state of *S. cerevisiae* characterized by respiratory repression and increased ethanol production which occurs under aerobic conditions and glucose levels above a certain limit (~0.2 g/L) [44]. Following glucose depletion (~11 h), a simultaneous decrease was detected in ethanol and acetate concentrations (Fig. 3D).

This finding, while preliminary, supports the hypothesis that *S. cerevisiae* exhibited diauxic growth, during which glucose is initially consumed followed by acetate and ethanol consumption. These results are consistent with those of previous research into the metabolism of *S. cerevisiae* which demonstrated the utilization ethanol and acetate as

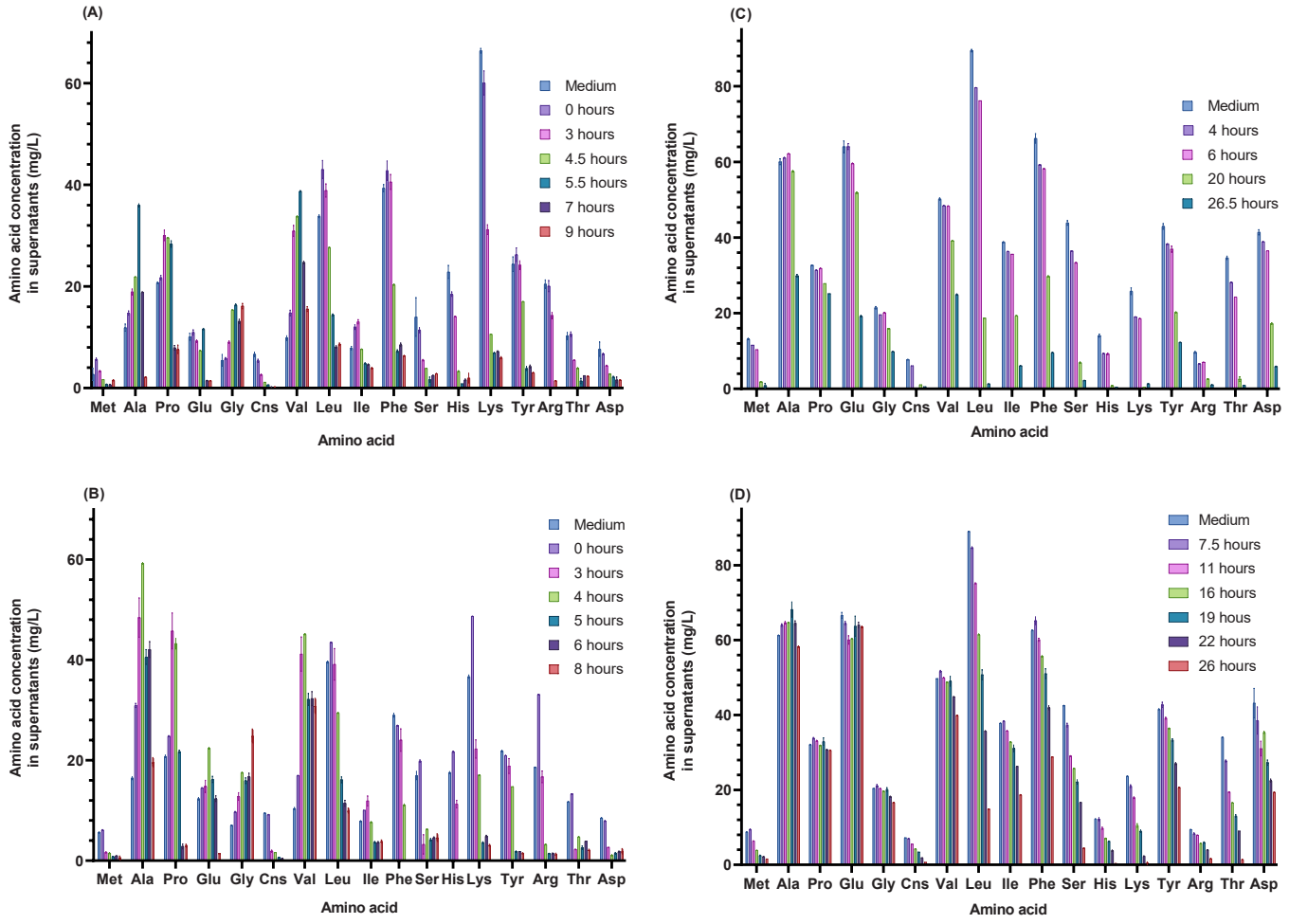

Fig. 4. Trends in amino acids concentrations (mg/L) during two repeated batch fermentations in bioreactor with Corynebacterium glutamicum (A, B) and Saccharomyces cerevisiae (C, D). Data is reported as meanSD of two technical replicates.

carbon sources after sugar depletion [39]. To fully understand the substrate utilization patterns in BSG hydrolysate-based medium, however, more analysis is required since *S. cerevisiae* is known to utilize a multitude of other C-sources including glycerol, maltose, fructose, sucrose, and galactose [39,45].

The amino acid profiles showed that Ala, Pro, Val, and Gly were the major amino acids produced by *C. glutamicum* (Figs. 4A and 4B). Production of these amino acids appeared to be growth-dependent as it increased with increasing OD_{600} values during the exponential phase. After 4–5 h, the production of Ala, Pro, and Val began to decline, while no general trend could be observed for Gly. The decreased productivity may be due to C-source limitation as both glucose and acetate were depleted after 4–5 h; therefore, supplementation with these substrates may promote prolonged and further growth and potentially increase amino acid production by *C. glutamicum*. All amino acids were consumed by *S. cerevisiae* during batch fermentation in bioreactor except for Ala which increased slightly during the first 10–11 h (Figs. 4C and 4D).

3.2.2.2. Fed-batch fermentation. As the amino acid production showed growth dependency for *C. glutamicum*, the effect of exponential feeding on amino acid production was investigated as a strategy to increase productivity. To do so, a fed-batch fermentation experiment was performed. It consisted of two phases: a batch phase and a fed-batch phase (Fig. S3). When the batch phase ended (9 h) (indicated by drop in off-gas CO_2), the fed-batch phase was initiated by exponential addition of feed composed of BSG hydrolysate and salts. To achieve high biomass concentration during the fed-batch phase, a higher glucose concentration (4 g/L) was used in the feed compared to the initial medium (2.5 g/L). It was evident from the growth curve that the culture was not growing exponentially during the fed-batch phase indicating a lack of some media components. Contrary to our expectations, besides Gly, all amino acid concentrations were found to be low throughout the fed-batch phase. The lack of amino acid production could be ascribed to an increase in amino acid consumption which may occur under C-limited conditions [46]. This hypothesis was corroborated by sample analysis indicating a complete depletion of glucose, acetate and lactate during the fed-batch phase. As it was observed in the batch phase, xylose and arabinose were not consumed during the fed-batch phase (data not shown). This suggested that *C. glutamicum* was not capable of metabolizing these sugars, even under severe nutrient deprivation (data not shown). Moreover, the observed accumulation of Gly could be due to selective degradation of intracellular proteins caused by nutrient limitation [47]. Further studies are needed to investigate the low or absent production of amino acids during the fed-batch phase. Future research should focus on optimizing the process conditions with emphasis on high feed substrate/glucose concentration as it seems to be the limiting factor. To maximize amino acid production, the feed composition and strategy should be optimized to match the desired growth rate during exponential feeding. This could potentially be achieved by increasing the concentrations of BSG-derived glucose in the feed which requires further optimization of the hydrolysis process. Such efforts should concentrate on optimizing the enzymatic hydrolysis of cellulose rather than hemicellulose since *C. glutamicum* did not utilize the hemicellulosic monosaccharides (xylose and arabinose) from the BSG hydrolysate

(Fig. S2). One possible strategy for optimizing enzymatic cellulose hydrolysis of BSG could be to remove hemicellulose and lignin using a sequential pretreatment with dilute acid and base as reported by Lee et al. [48]. This approach will lead to increased accessibility of cellulose to cellulases by removing the protective layers of lignin and hemicellulose. Alternatively, the use of deep eutectic solvents may be a facile and eco-friendly method for enhancing the enzymatic hydrolysis of cellulose because these solvents have been proven to efficiently extract lignin and hemicellulose under mild conditions [49].

3.3. Discussion

In the previous section, the overall performance of *S. cerevisiae* and *C. glutamicum* was compared with respect to growth, substrate consumption and amino acid production. This section will focus on the effect of cultivation mode (shake flask versus bioreactor) and growth phase on the production of selected amino acids by *S. cerevisiae* and *C. glutamicum*.

3.3.1. Effect of cultivation mode and growth phase on amino acid production

3.3.1.1. Corynebacterium glutamicum. The changes in Ala, Pro, Val and Gly concentrations observed during the exponential and stationary phases during batch fermentation in shake flasks and bioreactor with *C. glutamicum* are shown in Fig. 5. These results indicated that amino acid production depended on the growth phase and cultivation mode.

In the shake flask experiment, Ala, Pro and Val were produced during both growth phases whereas Gly was produced only during the stationary phase (Fig. 5A). The production of Ala, Pro and Val was higher in the stationary phase at 30 h (Ala: 193.6 mg/L, Pro: 20.3 mg/L, Val: 42.7 mg/L) compared to exponential phase (9 h) (Ala: 33.7 mg/L, Pro: 5.21 mg/L, Val: 32.3 mg/L). This was surprising as the productivity was expected to be higher during the exponential phase where the cells possessed the maximum metabolic activity. It is likely that the high amino acid concentrations observed in the stationary phase were caused by cell lysis rather than production as suggested by Paczia et al. [46].

During batch fermentation in bioreactor, the exponential phase (4 h) was associated with the highest production of Ala, Pro and Val (Ala: 42.8 mg/L, Pro: 22.4 mg/L, Val: 34.8 mg/L) (Fig. 5B). In the stationary phase (8 h), however, production of Ala and Val was lower than in the exponential phase while Pro was consumed (Ala: 3.16 mg/L, Pro: −17.8 mg/L, Val: 20.4 mg/L). For Gly, the opposite trend was observed as the production appeared to be higher in the stationary phase (Gly: 18.8 mg/L) and lower in the exponential phase (Gly: 10.5 mg/L).

Also, it was evident that Ala production during the stationary phase was substantially higher in shake flasks compared to bioreactor cultivation. This might be related to the different sample processing methods applied. In shake flask experiment, the cell-free supernatants were produced by centrifugation only, whereas in bioreactor experiments supernatants were obtained by a combination of centrifugation followed by filtration. As demonstrated by Paczia et al. [46], filtration could result in further loss of metabolites, which may explain the lower Ala production observed in the stationary phase of bioreactor cultivation.

3.3.1.2. Saccharomyces cerevisiae. The changes in amino acid concentrations observed during batch fermentation in shake flasks and bioreactor with *S. cerevisiae* are summarized in Fig. 6. In the shake flask experiments, Ala, Pro, Glu and Val were produced during the exponential phase (50 h). In contrast, Ala, Pro and Val were consumed while Glu was produced during the stationary phase (60 h) (Fig. 6A). Interestingly, the production Glu was nearly 50% higher during the stationary phase when compared to the exponential phase. As explained for Ala production by *C. glutamicum*, the high Glu concentration might be due to cell lysis.

Results from the bioreactor cultivations with *S. cerevisiae* showed a clear absence of amino acid production except for Ala which increased slightly during the exponential phase (Fig. 6B). One plausible explanation for this could be that the initial pH values differed between the shake flask (pH 6.5) and the bioreactor (pH 5.0) cultivations. Besides medium pH, the two cultivation modes were different with regards to agitation type, aeration and scaling. It was therefore likely that variations in amino acid production were caused by a combination of several parameters rather than the single effect of medium pH. Further studies are required to investigate the effect of pH on amino acid production by *S. cerevisiae*. Furthermore, amino acid production by *S. cerevisiae* may be enhanced by increasing the salinity as demonstrated by Malaney et al. [33] who found elevated extracellular concentrations of certain amino acids at high NaCl concentrations.

3.3.2. General discussion

Although promising, the fermentation processes presented here were not without limitations. First, the productivities were considerably lower compared to those reported for industrial production of amino acids by fermentation. Lee et al. [50] reported production of 98 g/L Ala, 150 g/L Val, and 13 g/L Pro from large-scale fermentations with

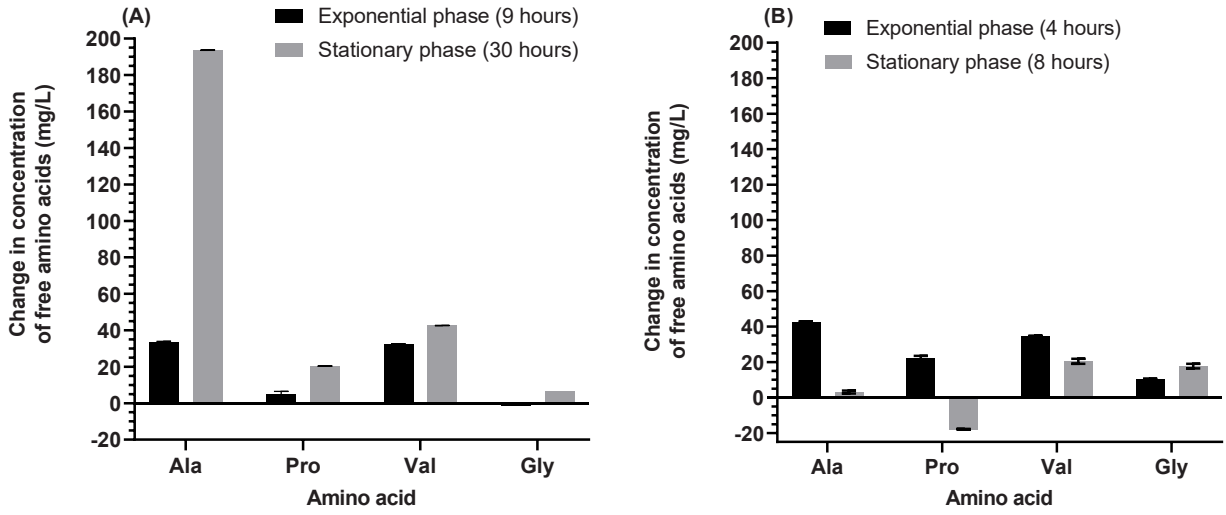

Fig. 5. Effect of cultivation mode and growth phase on amino acid production by Corynebacterium glutamicum during batch fermentation in (A): shake flasks and (B): bioreactor.

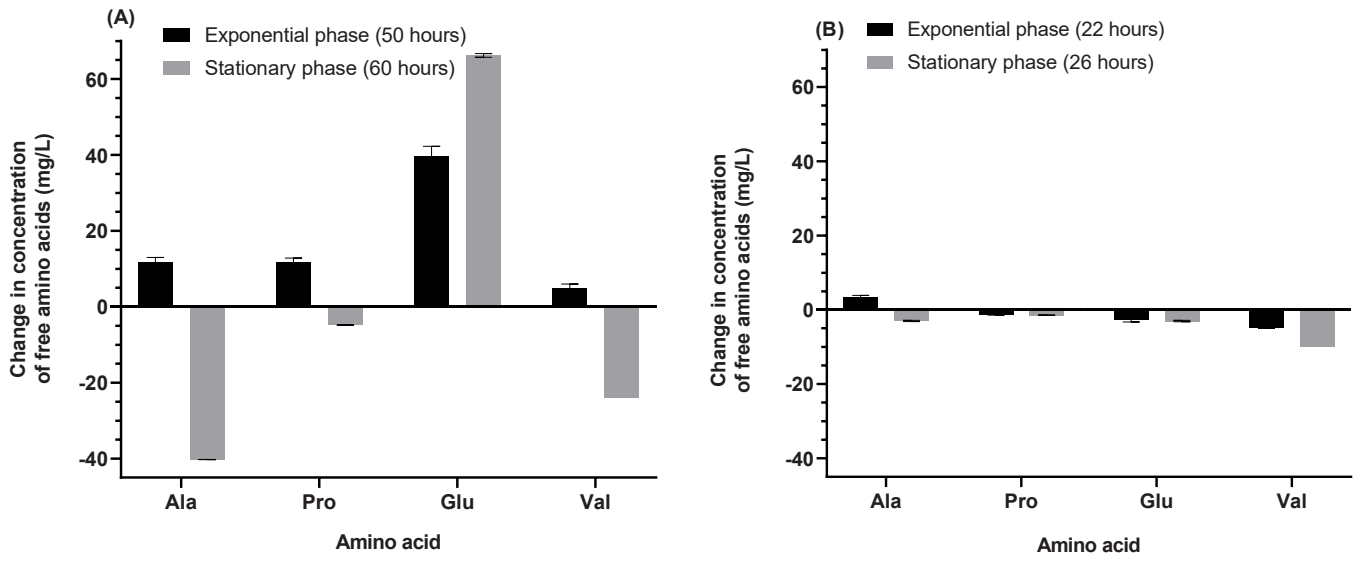

Fig. 6. Effect of cultivation mode and growth phase on amino acid production by Saccharomyces cerevisiae during batch fermentation in (A): shake flasks and (B): bioreactor.

metabolically engineered *C. glutamicum* strains. In the present study, a native strain of *C. glutamicum* was employed which yielded 0.043 g/L Ala, 0.035 g/L Val, and 0.022 g/L Pro when cultured in a BSG hydrolysate-based medium. This difference may be related to the properties of the producing strain since industrial processes typically employ strains that have been metabolically engineered to overproduce certain amino acids. Another reason could be the presence of lignocellulose-derived inhibitors originating from the hydrolysis of BSG. Recent research has suggested that compounds derived from lignocellulose, such as furfural, 5-hydroxymethylfurfural (HMF), vanillin, syringaldehyde, and acetate, inhibit the production of value-added compounds by *C. glutamicum* including ethanol, succinic acid, and amino acids [51–53]. As proposed by Wang et al. [54], this issue could be overcome by using strains exhibiting high inhibitor tolerance. Inhibitory effects caused by acetate could also be avoided by performing the hydrolysis in a different buffer solution as the ammonium acetate buffer may be the main contributing factor for the high acetate media concentrations (8–9 g/L). Second, *C. glutamicum* did not produce any Glu. This finding was surprising as *C. glutamicum* is the most studied producer of Glu. The absence of Glu accumulation may be due to excessive biotin levels in the hydrolysate. High biotin levels are associated with reduced C-flux towards Glu synthesis and strengthened cell walls resulting in reduced secretion of Glu to the medium [55]. Even though biotin was not quantified in the present study, it is reasonable to assume that the BSG hydrolysate contained significant biotin concentrations as Karlović et al. [56] reported a biotin content of ~100 μg/kg dry weight of BSG. While Glu secretion can be induced under biotin-rich conditions by the addition of antibiotics to inhibit cell wall synthesis, this poses environmental and health challenges and should be avoided [54,55]. A better approach to tackle this issue was described by Wen and Bao [57] who showed that high Glu production can be achieved by using a metabolically engineered strain of *C. glutamicum* designed to overproduce it under biotin-rich conditions. Together, these strategies may successfully optimize Glu production from BSG by *C. glutamicum*. With respect to *S. cerevisiae*, a comparably high Glu production was found in the shake flask experiment. This finding suggests that Glu production by *S. cerevisiae* was not affected by biotin.

Evaluation of other amino acid-producing microorganisms would be a viable topic for future research. Besides *E. coli*, *C. glutamicum*, *L. plantarum* and *S. cerevisiae*, certain *Bacillus* species have been reported to be excellent amino acid-producers [58]. Previous studies have demonstrated the fermentative production of several amino acids by *B. cereus*, *B. anthracis* and *B. spp*. using either pure glucose or molasses as substrates [58,59]. No prior studies have yet considered the utilization of lignocellulosic feedstock as substrates for amino acid production by *Bacillus* species. Therefore, a future study investigating amino acid production from BSG by different *Bacillus* species would be interesting.

4. Conclusions

The use of brewer's spent grain-derived lignocellulosic hydrolysate as a fermentation substrate for amino acid production using selected microorganisms was demonstrated for the first time. *Corynebacterium glutamicum* and *Saccharomyces cerevisiae* were selected for amino acid production. Cultivation mode and growth phase affected amino acid production in both microorganisms. In shake flasks and bioreactor, *C. glutamicum* produced alanine, proline, valine, and glycine where highest production was found for alanine in shake flasks. *S. cerevisiae* produced alanine, proline, valine and glutamic acid in shake flasks, with highest production of glutamic acid. In bioreactor, *S. cerevisiae* did not produce amino acids.

CRediT authorship contribution statement

Freja Karlsen: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft preparation, Investigation, Visualization. **Sushil S. Gaykawad**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing—review and editing. **Catherine Boccadoro**: Conceptualization, Writing – review & editing, Project administration, Funding acquisition. **Peter V. Skov**: Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that has been used is confidential.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bej.2023.109059.

References

[1] G. Zhang, X. Ren, X. Liang, Y. Wang, D. Feng, Y. Zhang, M. Xian, H. Zou, Improving the microbial production of amino acids: from conventional approaches to recent trends, Biotechnol. Bioprocess Eng. 26 (2021) 708–727, https://doi.org/10.1007/s12257-020-0390-1.

[2] S. Sanchez, R. Rodríguez-Sanoja, A. Ramos, A.L. Demain, Our microbes not only produce antibiotics, they also overproduce amino acids, J. Antibiot. (Tokyo) 71 (2018) 26–36, https://doi.org/10.1038/ja.2017.142.

[3] Amino Acids Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2023–2028. Available at: https://www.researchandmarkets.com/report/amino-acids. (Accessed 7 August 2023).

[4] A. Stoimenova, K. Ivanov, D. Obreshkova, L. Saso, Biotechnology in the production of pharmaceutical industry ingredients: Amino acids, Biotechnol. Biotechnol. Equip. 27 (2013) 3620–3626, https://doi.org/10.5504/bbeq.2012.0134.

[5] C.J. Toe, H.L. Foo, T.C. Loh, R. Mohamad, R.A. Rahim, Z. Idrus, Extracellular proteolytic activity and amino acid production by lactic acid bacteria isolated from malaysian foods, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20071777.

[6] M. D'Este, M. Alvarado-Morales, I. Angelidaki, Amino acids production focusing on fermentation technologies – a review, Biotechnol. Adv. 36 (2018) 14–25, https://doi.org/10.1016/j.biotechadv.2017.09.001.

[7] D. Glittenberg, Starch-Based Biopolymers in Paper, Corrugating, and Other Industrial Applications, Elsevier B.V, 2012, https://doi.org/10.1016/B978-0-444-53349-4.00258-2.

[8] B. Zhang, Y. Jiang, Z. Li, F. Wang, X.Y. Wu, Recent progress on chemical production from non-food renewable feedstocks using corynebacterium glutamicum, Front. Bioeng. Biotechnol. 8 (2020) 1–11, https://doi.org/10.3389/fbioe.2020.606047.

[9] S.I. Mussatto, G. Dragone, I.C. Roberto, Brewers' spent grain: generation, characteristics and potential applications, J. Cereal Sci. 43 (2006) 1–14, https://doi.org/10.1016/j.jcs.2005.06.001.

[10] S. Mitri, S.J. Salameh, A. Khelfa, E. Leonard, R.G. Maroun, N. Louka, M. Koubaa, Valorization of brewers' spent grains: pretreatments and fermentation, a review, Fermentation 8 (2022), https://doi.org/10.3390/fermentation8020050.

[11] S. Aliyu, M. Bala, Brewer's spent grain: a review of its potentials and applications, Afr. J. Biotechnol. 10 (2011) 324–331, https://doi.org/10.5897/AJBx10.006.

[12] F. Karlsen, I. Lund, P.V. Skov, Optimisation of alkaline extraction of protein from brewer's spent grain, J. Inst. Brew. 128 (2022) 150–161, https://doi.org/10.1002/jib.703.

[13] C. Wen, J. Zhang, Y. Duan, H. Zhang, H. Ma, A mini-review on brewer's spent grain protein: isolation, physicochemical properties, application of protein, and functional properties of hydrolysates, J. Food Sci. 84 (2019) 3330–3340, https://doi.org/10.1111/1750-3841.14906.

[14] S.I. Mussatto, M. Fernandes, Lignin recovery from brewer ' s spent grain black liquor, Carbohydr. Polym. 70 (2007) 218–223, https://doi.org/10.1016/j.carbpol.2007.03.021.

[15] A.C. Cassoni, P. Costa, I. Mota, M.W. Vasconcelos, M. Pintado, Recovery of lignins with antioxidant activity from Brewer's spent grain and olive tree pruning using deep eutectic solvents, Chem. Eng. Res. Des. 192 (2023) 34–43, https://doi.org/10.1016/j.cherd.2023.01.053.

[16] G. Mandalari, C.B. Faulds, A.I. Sancho, A. Saija, G. Bisignano, R. Locurto, K. W. Waldron, Fractionation and characterisation of arabinoxylans from brewers' spent grain and wheat bran, J. Cereal Sci. 42 (2005) 205–212, https://doi.org/10.1016/j.jcs.2005.03.001.

[17] L. Casas-Godoy, J.L. González-Escobar, A.G. Mathis, I. Barrera-Martínez, Revalorization of untreated Brewer's spent grain: novel and versatile feedstock to produce cellulases, lipases, and yeast biomass in a biorefinery approach, Biomass-.-. Convers. Biorefinery. 13 (2023) 1659–1670, https://doi.org/10.1007/s13399-020-01157-3.

[18] M. Hashemi, S.H. Razavi, S.A. Shojaosadati, S.M. Mousavi, The potential of brewer's spent grain to improve the production of α-amylase by Bacillus sp. KR-8104 in submerged fermentation system, N. Biotechnol. 28 (2011) 165–172, https://doi.org/10.1016/j.nbt.2010.10.009.

[19] D. Sousa, A. Venâncio, I. Belo, J.M. Salgado, Mediterranean agro-industrial wastes as valuable substrates for lignocellulolytic enzymes and protein production by solid-state fermentation, J. Sci. Food Agric. 98 (2018) 5248–5256, https://doi.org/10.1002/jsfa.9063.

[20] S. Shindo, T. Tachibana, Production of L-lactic acid from spent grain, a by-product of beer production, J. Inst. Brew. 110 (2004) 347–351, https://doi.org/10.1002/j.2050-0416.2004.tb00631.x.

[21] T.O. Femi-Ola, V.A. Atere, Citric acid production from brewers spent grain by Aspergillus niger and Saccharomyces cerevisiae, Int. J. Res. Biosci. 2 (2013) 30–36.

[22] A. Rojas-chamorro, I. Romero, C.L. Juan, E. Castro, Brewer ' s spent grain as a source of renewable fuel through optimized dilute acid pretreatment 148 (2020) 81–90, https://doi.org/10.1016/j.renene.2019.12.030.

[23] P.J. Van Soest, R.W. McQueen, Symposium on fibre in human nutrition, the chemistry and estimation of fibre, Proc. Nutr. Soc. 32 (1973).

[24] A.M. Magomya, D. Kubmarawa, J.A. Ndahi, G.G. Yebpella, Determination of plant proteins via the kjeldahl method and amino acid analysis: A Comparative study, Int. J. Sci. Technol. Res. 3 (2014) 68–72.

[25] P. Forssell, H. Kontkanen, H.A. Schols, S. Hinz, V.G.H. Eijsink, J. Treimo, J. A. Robertson, K.W. Waldron, C.B. Faulds, J. Buchert, Hydrolysis of brewers' spent grain by carbohydrate degrading enzymes, J. Inst. Brew. 114 (2008) 306–314, https://doi.org/10.1002/j.2050-0416.2008.tb00774.x.

[26] A. Venkata Narayana, A. Vamsi Priya, R. Venkata Nadh, A.V.N. Swami, B. Sumalatha, M. Vijaya Leela, Methionine production by coryneform bacteria through fermentation, Res. J. Pharm. Biol. Chem. Sci. 4 (2013) 1489–1498.

[27] L.M. Blank, B.E. Ebert, B. Bühler, A. Schmid, Metabolic capacity estimation of Escherichia coli as a platform for redox biocatalysis: Constraint-based modeling and experimental verification, Biotechnol. Bioeng. 100 (2008) 1050–1065, https://doi.org/10.1002/bit.21837.

[28] L.F. Coelho, C.J.B. De Lima, C.M. Rodovalho, M.P. Bernardo, J. Contiero, Lactic acid production by new lactobacillus plantarum LMISM6 grown in molasses: Optimization of medium composition, Braz. J. Chem. Eng. 28 (2011) 27–36, https://doi.org/10.1590/S0104-66322011000100004.

[29] P. Van Hoek, J.P. Van Dijken, J.T. Pronk, Effect of specific growth rate on fermentative capacity of baker's yeast, Appl. Environ. Microbiol. 64 (1998) 4226–4233, https://doi.org/10.1128/aem.64.11.4226-4233.1998.

[30] B. Blombach, T. Riester, S. Wieschalka, C. Ziert, J.W. Youn, V.F. Wendisch, B. J. Eikmanns, Corynebacterium glutamicum tailored for efficient isobutanol production, Appl. Environ. Microbiol. 77 (2011) 3300–3310, https://doi.org/10.1128/AEM.02972-10.

[31] S.A. Cohen, K.M. De Antonis, Applications of amino acid derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. Analysis of feed grains, intravenous solutions and glycoproteins, J. Chromatogr. A. 661 (1994) 25–34, https://doi.org/10.1016/0021-9673(93)E0821-B.

[32] V. Gopinath, K.M. Nampoothiri, Corynebacterium Glutamicum. Second Edi, Elsevier, 2014, https://doi.org/10.1016/B978-0-12-384730-0.00076-8.

[33] G.W. Malaney, R.D. Tanner, A.M. Rodrigues, The production of extracellular and intracellular free amino acids during aerated fermentation of glucose by Baker's yeast (Saccharomyces cerevisiae), Folia Microbiol. 36 (1991) 468–477, https://doi.org/10.1007/BF02884068.

[34] A. Rodriguez, J.A. Martínez, N. Flores, A. Escalante, G. Gosset, F. Bolivar, Engineering Escherichia coli to overproduce aromatic amino acids and derived compounds, Microb. Cell Fact. 13 (2014) 1–15, https://doi.org/10.1186/s12934-014-0126-z.

[35] E. Wagner, E. Sierra-Ibarra, N.L. Rojas, A. Martinez, One-pot bioethanol production from brewery spent grain using the ethanologenic Escherichia coli MS04, Renew. Energy 189 (2022) 717–725, https://doi.org/10.1016/j.renene.2022.03.014.

[36] Y. Assefa, S. Anuradha Jabasingh, Lactic acid production from brewer's spent grain by Lactobacillus plantarum ATCC 8014, J. Sci. Ind. Res. (India). 79 (2020) 610–613, https://doi.org/10.56042/jsir.v79i7.40473.

[37] X. Liu, B. Jia, X. Sun, J. Ai, L. Wang, C. Wang, F. Zhao, J. Zhan, W. Huang, Effect of Initial PH on growth characteristics and fermentation properties of saccharomyces cerevisiae, J. Food Sci. 80 (2015) M800–M808, https://doi.org/10.1111/1750-3841.12813.

[38] M. Follmann, I. Ochrombel, R. Krämer, C. Trötschel, A. Poetsch, C. Rückert, A. Hüser, M. Persicke, D. Seiferling, J. Kalinowski, K. Marin, Functional genomics of pH homeostasis in corynebacterium glutamicum revealed novel links between pH response, oxidative stress, iron homeostasis and methionine synthesis, BMC Genom. 10 (2009), https://doi.org/10.1186/1471-2164-10-621.

[39] W. Estela-Escalante, M. Rychtera, K. Melzoch, B. Hatta-Sakoda, Effect of aeration on the fermentative activity of saccharomyces cerevisiae cultured in apple juice, Rev. Mex. Ing. Quim. 11 (2012) 211–226.

[40] N.M.M. Al-Mhanna, Observation of Crabtree effect and diauxic behaviour of yeast by using absorption, Chem. Eng. Trans. 21 (2010) 1465–1470, https://doi.org/10.3303/CET1021245.

[41] G. Stahl, S.N.B. Salem, L. Chen, B. Zhao, P.J. Farabaugh, Translational accuracy during exponential, postdiauxic, and stationary growth phases in Saccharomyces cerevisiae, Eukaryot. Cell. 3 (2004) 331–338, https://doi.org/10.1128/EC.3.2.331-338.2004.

[42] E. Coelho, M.A.M. Rocha, A.S.P. Moreira, M.R.M. Domingues, M.A. Coimbra, Revisiting the structural features of arabinoxylans from brewers' spent grain, Carbohydr. Polym. 139 (2016) 167–176, https://doi.org/10.1016/j.carbpol.2015.12.006.

[43] R. Gerstmeir, V.F. Wendisch, S. Schnicke, H. Ruan, M. Farwick, D. Reinscheid, B. J. Eikmanns, Acetate metabolism and its regulation in Corynebacterium glutamicum, J. Biotechnol. 104 (2003) 99–122, https://doi.org/10.1016/S0168-1656(03)00167-6.
[44] E. Postma, C. Verduyn, W.A. Scheffers, J.P. Van Dijken, Enzymic analysis of the crabtree effect in glucose-limited chemostat cultures of Saccharomyces cerevisiae, Appl. Environ. Microbiol. 55 (1989) 468–477, https://doi.org/10.1128/aem.55.2.468-477.1989.
[45] B. Turcotte, X.B. Liang, F. Robert, N. Soontorngun, Transcriptional regulation of nonfermentable carbon utilization in budding yeast, FEMS Yeast Res 10 (2010) 2–13, https://doi.org/10.1111/j.1567-1364.2009.00555.x.
[46] N. Paczia, A. Nilgen, T. Lehmann, J. Gätgens, W. Wiechert, S. Noack, Extensive exometabolome analysis reveals extended overflow metabolism in various microorganisms, Microb. Cell Fact. 11 (2012) 1–14, https://doi.org/10.1186/1475-2859-11-122.
[47] E. Gur, M. Korman, N. Hecht, O. Regev, S. Schlussel, N. Silberberg, Y. Elharar, How to control an intracellular proteolytic system: Coordinated regulatory switches in the mycobacterial Pup-proteasome system, Biochim. Biophys. Acta - Mol. Cell Res. 1864 (2017) 2253–2260, https://doi.org/10.1016/j.bbamcr.2017.08.012.
[48] J.W. Lee, J.Y. Kim, H.M. Jang, M.W. Lee, J.M. Park, Sequential dilute acid and alkali pretreatment of corn stover: Sugar recovery efficiency and structural characterization, Bioresour. Technol. 182 (2015) 296–301, https://doi.org/10.1016/j.biortech.2015.01.116.
[49] C.W. Zhang, S.Q. Xia, P.S. Ma, Facile pretreatment of lignocellulosic biomass using deep eutectic solvents, Bioresour. Technol. 219 (2016) 1–5, https://doi.org/10.1016/j.biortech.2016.07.026.
[50] J.Y. Lee, Y.A. Na, E. Kim, H.S. Lee, P. Kim, The actinobacterium Corynebacterium glutamicum, an industrial workhorse, J. Microbiol. Biotechnol. 26 (2016) 807–822, https://doi.org/10.4014/jmb.1601.01053.
[51] S. Sakai, Y. Tsuchida, S. Okino, O. Ichihashi, H. Kawaguchi, T. Watanabe, M. Inui, H. Yukawa, Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested Corynebacterium glutamicum R, Appl. Environ. Microbiol. 73 (2007) 2349–2353, https://doi.org/10.1128/AEM.02880-06.
[52] H.T. Xu, C. Wang, Z.H. Zhou, Z.J. Chen, H. Cai, Effects of lignocellulose-derived inhibitors on growth and succinic acid accumulation by Corynebacterium glutamicum, Biotechnol. Bioprocess Eng. 20 (2015) 744–752, https://doi.org/10.1007/s12257-015-0201-2.
[53] X. Wang, I. Khushk, Y. Xiao, Q. Gao, J. Bao, Tolerance improvement of Corynebacterium glutamicum on lignocellulose derived inhibitors by adaptive evolution, Appl. Microbiol. Biotechnol. 102 (2018) 377–388, https://doi.org/10.1007/s00253-017-8627-4.
[54] X. Wang, I. Khushk, Y. Xiao, Q. Gao, J. Bao, Tolerance improvement of Corynebacterium glutamicum on lignocellulose derived inhibitors by adaptive evolution, Appl. Microbiol. Biotechnol. 102 (2018) 377–388, https://doi.org/10.1007/s00253-017-8627-4.
[55] J. Wen, Y. Xiao, T. Liu, Q. Gao, J. Bao, Rich biotin content in lignocellulose biomass plays the key role in determining cellulosic glutamic acid accumulation by Corynebacterium glutamicum, Biotechnol. Biofuels. 11 (2018) 1–13, https://doi.org/10.1186/s13068-018-1132-x.
[56] A. Karlović, A. Jurić, N. Ćorić, K. Habschied, V. Krstanović, K. Mastanjević, By-products in the malting and brewing industries-re-usage possibilities, Fermentation 6 (2020) 1–17, https://doi.org/10.3390/FERMENTATION6030082.
[57] J. Wen, J. Bao, Engineering Corynebacterium glutamicum triggers glutamic acid accumulation in biotin-rich corn stover hydrolysate, Biotechnol. Biofuels. 12 (2019) 1–11, https://doi.org/10.1186/s13068-019-1428-5.
[58] F.R. Shakoori, A.M. Butt, N.M. Ali, M.T. Zahid, A. Rehman, A.R. Shakoori, Optimization of fermentation media for enhanced amino acids production by bacteria isolated from natural sources, Pak. J. Zool. 44 (2012) 1145–1157.
[59] K. Abou-taleb, Enhancing production of amino acids from bacillus spp. using batch and fed-batch fermentation strategies, Br. Microbiol. Res. J. 5 (2015) 257–272, https://doi.org/10.9734/bmrj/2015/12447.