Development of chemically defined media supporting high cell density growth of *Ketogulonicigenium vulgare* and *Bacillus megaterium*

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**A B S T R A C T**

The immediate precursor of L-ascorbic acid, or vitamin C, is 2-keto-1-gulonic acid (2-KLG). This is commonly produced commercially by *Ketogulonicigenium vulgare* and *Bacillus megaterium*, using corn steep liquor powder (CSLP) as an organic nitrogen source. In this study, the effects of the individual CSLP components (amino acids, vitamins, and metal elements) on 2-KLG production were evaluated, with the aim of developing a complete, chemically defined medium for 2-KLG production. Forty components of CSLP were analyzed, and key components were correlated to biomass, 2-KLG productivity, and consumption rate of L-sorbose. Glycine had the greatest effect, followed by serine, biotin, proline, nicotinic acid, and threonine. The combination of 0.28 g L⁻¹ serine, 0.36 g L⁻¹ glycine, 0.18 g L⁻¹ threonine, 0.28 g L⁻¹ proline, 0.19 g L⁻¹ nicotinic acid, and 0.62 mg L⁻¹ biotin in a chemically defined medium produced the highest maximum biomass concentration (4.2 × 10⁹ cfu ml⁻¹), 2-KLG concentration (58 g L⁻¹), and yield (0.76 g g⁻¹) after cultivating for 28 h.

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1. Introduction

With an estimated global production of 110,000 tons per year, the market for L-ascorbic acid, commonly known as vitamin C, is becoming increasingly more competitive (Macauley et al., 2001). The well-known two-step production process, originally developed in China in the 1960’s (Yin et al., 1997), was the first unique process to compete with the previously employed Reichstein process and is currently used by all Chinese manufacturers. In the two-step fermentation process, *Ketogulonicigenium vulgare* (previously identified as *Glucobacter oxydans*) (Yang et al., 2006) and *Bacillus megaterium* are used to synthesize the 1-ascorbic acid precursor 2-keto-1-gulonic acid (2-KLG). A high 2-KLG concentration (75.8 g L⁻¹) and yield (94.8%) can be achieved by providing 1-sorbose to *K. vulgare* and *B. megaterium* during 72 h of cultivation (Xu et al., 2004), with the 2-KLG yield significantly increased by the use of two bacterial strains, rather than just *K. vulgare* (Feng et al., 2000). *B. megaterium* is generally thought to act as a companion strain that synthesizes and secretes metabolites into the fermentation broth, stimulating the growth of *K. vulgare* and increasing the 2-KLG production (Zhao et al., 2008). Recently, genome sequences for *K. vulgare* and *B. megaterium* have enabled the development of metabolic models and opened up novel “omics” strategies for examining cellular metabolism during fermentation. All of these strategies are greatly facilitated by the use of a chemically-defined medium, in which metabolism is more easily defined.

As it lacks various biosynthetic pathways, *K. vulgare* generally requires a medium rich in nutrients. A nutrient-rich environment can be provided by a semi-defined medium (SDM), formulated mostly with defined chemicals (except for corn steep liquor powder (CSLP)) (Takagi et al., 2009). However, using an SDM in physiological studies focusing on metabolism and regulation makes data more difficult to interpret as consumption of the numerous intermediary metabolites produced during biosynthesis of macromolecules is not easily quantified. For this reason, a chemically defined medium (CDM) that supports reasonable cell growth can be of great help in the study of gene regulation and metabolic fluxes. By systematically adding or removing components from the CDM formulation, the specific nutritional and regulatory requirements for growth and targeted metabolic pathways can be determined. Uncertainties due to the complicated interactions among complex components can be minimized or at least more easily understood, and the culture environment is more reproducible. In this study, 2-KLG fermentation by *K. vulgare* and *B. megaterium* is used as a model for investigating the qualitative
physiological role of CSLP composition on metabolic behaviors under conditions of industrial fermentation. In addition, a CDM was developed that supports growth of *K. vulgare* and *B. megaterium* that is comparable to or exceeds growth on a semi-defined medium.

2. Methods

2.1. Bacterial strains and media

The strains of *K. vulgare* and *B. megaterium* used in this study were obtained from Jiangsu Jiangshan Pharmaceutical Co. Ltd., and stored at the Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University.

Medium A for seed cultures contained (g L\(^{-1}\)): L-sorbose 20, yeast extract 3, peptone 10, beef extract 3, CSLP 1.5, urea 1, CaCO\(_3\) 1, MgSO\(_4\) 7H\(_2\)O 0.2, KH\(_2\)PO\(_4\) 1. For plate cultures, 2% agar was added. Medium B for fermentation contained (g L\(^{-1}\)): L-sorbose 80, urea 12, CSLP 5, MgSO\(_4\) 7H\(_2\)O 0.1, KH\(_2\)PO\(_4\) 1. Synthetic medium C contained (g L\(^{-1}\)): L-sorbose 80, urea 12, MgSO\(_4\) 7H\(_2\)O 0.1, KH\(_2\)PO\(_4\) 1, aspartate 0.10, glutamate 0.26, histidine 0.05, arginine 0.05, alanine 0.19, tyrosine 0.03, cysteine 0.01, valine 0.10, methionine 0.04, phenylalanine 0.06, isoleucine 0.06, leucine 0.16, lysine 0.05; metal ion solutions 1 mL L\(^{-1}\), vitamin solutions 0.1 mL L\(^{-1}\), pH 6.7–7.0. The concentrations of serine, glycine, threonine, proline, nicotinic acid, and biotin were adjusted according to the experimental design.

Metal ion solutions were (g L\(^{-1}\)): ZnCl\(_2\) 1.75, FeCl\(_3\) 4.61, MnCl\(_2\) 0.85, CuCl\(_2\) 0.06, KCl 442.60, NaCl 44.71, CaCl\(_2\) 17.39, KH\(_2\)PO\(_4\) 148.68. Vitamin solutions were (g L\(^{-1}\)): vitamin B1 0.04, vitamin B2 0.30, vitamin B6 1.20, vitamin B12 0.06, pantothenic acid 1.15, and folic acid 0.01.

In culture flasks, 5 g L\(^{-1}\) CaCO\(_3\) were added to buffer the fermentation broth. The initial pH of all media was adjusted to 7.0. L-sorbose and CaCO\(_3\) were sterilized separately prior to adding to the medium.

2.2. Culture conditions

The seed culture inoculated from a slant was cultivated at 30 °C, 200 rpm, in a 750-mL flask containing 75 mL medium A on a reciprocal shaker for 32 h (*K. vulgare*) or 9 h (*B. megaterium*). The seeds were mixed and cultured for another 18 h, and then the mixture of *K. vulgare* and *B. megaterium* was transferred into the fermentation medium (Zhang et al., 2010). Fermentations were carried out in 750-mL flasks containing 75 mL medium B or in a 7-L jar fermentor (KF-7 L, Korea Fermentor Co., Inchon, Korea) with 4 L of medium B. The amount of inoculum was 10% (v/v). Culture flasks were incubated for 68 h at 200 rpm. In fermentor cultures, the pH was automatically controlled to 7.0 with 8 M NaOH solution, with stirring at 400 rpm and air flow of 1.5 L min\(^{-1}\). All experiments were performed in triplicate. All cultivations were at 30 °C (Takagi et al., 2009).

2.3. CSLP sample

The 18 CSLP samples were collected from eight different habitats (Shandong (8), Hubei (3), Henan (2), Shanghai (1), Jiangsu (1), Hebei (1), Sichuan (1), and Jiangxi (1)) in China; further details are given in Table 1.

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<th>No.</th>
<th>Manufacturer Source</th>
<th>Source</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>2</td>
<td>Wuhan Galaxy Chemical Co., Ltd</td>
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<td>5</td>
<td>Binzhou Gangfa Biotechnology Co., Ltd.</td>
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<td>6</td>
<td>Shandong Fulidai Biotechnology Co., Ltd.</td>
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</tr>
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<td>7</td>
<td>Wuhan Bioco Sci. &amp; Tech. Dev. Co., Ltd</td>
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<tr>
<td>8</td>
<td>Wuhan Jiabao Sugar Co., Ltd</td>
<td>Wuhan, Hubei, China</td>
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<td>9</td>
<td>Shandong Douping Hebshan Biotechnology Co., Ltd.</td>
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<td>18</td>
<td>Jiangxi Jinbiyuan Industrial Co., Ltd.</td>
<td>Nanchang, Jiangxi, China</td>
</tr>
</tbody>
</table>

Table 1 Eighteen different samples of CSLP.

![Fig. 1. Fermentation parameters from different CSLP batches. (A) Biomass, (B) 2-KLG productivity, (C) Rate of L-sorbose consumption.](image-url)
2.4. Detection of CSLP components

2.4.1. Amino acid concentration determination
CSLP (0.2–0.25 g) was placed in 15-mL ampoules with 8 mL of 6.0 M HCl. Ampoules were vacuum-sealed and hydrolyzed at 110°C for 24 h, then 1 mL of hydrolysate was withdrawn and evaporated to dryness under vacuum at 45°C to remove HCl. Hydrolysate was dissolved in 5 mL of 0.02 M HCl, centrifuged at 5000 rpm and filtered, and 1 mL of supernatant was used for amino acid analysis, using pre-column ortho-phthalaldehyde and fluorenylmethyl chloroformate (for proline) derivatization (Chen et al., 2007).

Amino acids were separated by Agilent 1100 high-pressure liquid chromatography (HPLC) (Agilent, USA) using a 4.0 × 125-mm Hypersil ODS C18 column, with solvents and gradient conditions as previously reported (Chen et al., 2007). Detection wavelengths were set at UV 338 nm, or 262 nm for proline. The identities and quantities of amino acids were assessed by comparison to retention times and peak areas of amino acid standards (Chen et al., 2007).

2.4.2. Vitamin and metal concentration determinations
Vitamin B1, vitamin B2, vitamin C, nicotinic acid, vitamin B6, vitamin B12, pantothenic acid, folic acid, and free biotin were determined by HPLC (Heudi et al., 2005).

The levels of chromium (Cr), cadmium (Cd), lead (Pb), manganese (Mn), and copper (Cu) were determined by graphite furnace atomic absorption spectrophotometry (Hallen et al., 1995; Lin and Huang, 2001). Zinc (Zn), iron (Fe), magnesium (Mg), calcium (Ca), arsenic (As), and selenium (Se) were determined by flame atomic absorption spectrometry (Bermejo-Barrera et al., 2000; Matusiewicz and Krawczyk, 2006). Potassium (K) and sodium (Na) were determined by flame atomic emission spectrometry. Phosphorus was determined by molybdenum blue spectrophotometry (Korenaga and Sun, 1996).

2.5. Analytical methods for the substrate, metabolites, and biomass
L-sorbose and 2-KLG were quantified by HPLC. Samples were prepared by 1:10 dilution in the solvent mobile phase (1.1 mL ACS-grade sulfuric acid diluted to 4 L using Milli-Q water), followed by filtration through a 0.45-μm porous membrane (Urbance et al., 2001). Cell concentrations were measured by optical density at 660 nm after dissolving calcium carbonate in 0.1 mol L⁻¹ HCl. *K. vulgare* and *B. megaterium* were enumerated by hemocytometer count and confirmed by standard plate count. To determine dry cell mass, cultures were centrifuged at 10,000 rpm at 4°C for 5 min, and the pellet was dried overnight at 105°C. The CFU density value was converted to dry cell weight (DCW) using the relationship that a CFU value of 1 × 10⁹ is equal to 0.93 g DCW L⁻¹ (*K. vulgare*) and 8.27 g DCW L⁻¹ (*B. megaterium*).

For carbon balance calculations, the elemental composition of *K. vulgare* was assumed to be C₅H₇NO₂. The carbon balance was calculated as follows: carbon balance (%) = output total carbon (total moles of carbon contained in the biomass and 2-KLG)/input total carbon (moles of sorbose consumed).

2.6. Statistical analysis
Pearson linear correlation analysis was used to determine significant linear correlations between the parameters. Linear correlations were considered significant at a P < 0.05. SPSS 16.0 software (SPSS, Chicago, IL, USA) was used for statistical analyses. The degree of variability was estimated by the coefficient of variation (CV) for the 18 different CSLP samples.

3. Results and discussion

3.1. Effect of CLSP on 2-KLG production by *K. vulgare* and *B. megaterium*
Under identical nutrimental and environmental conditions, 18 sources of CSLP from different production batches were used as

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**Fig. 2.** The concentrations of the main components in 18 CSLP batches. Each symbol within a column represents one concentration for each of the 18 batches. (A) Amino acids – open circles, (B) Vitamins – open diamonds, (C) Metal elements – Xs. In each graph, the line links the mean of each CSLP component for the 18 batches. Legend: D, aspartate; E, glutamate; S, serine; H, histidine; G, glycine; T, threonine; R, arginine; A, alanine; Y, tyrosine; C, cysteine; V, valine; M, methionine; F, phenylalanine; I, isoleucine; L, leucine; KL, lysine; PR, proline; B1, vitamin B1; B2, vitamin B2; VC, vitamin C; NA, nicotinic acid; B6, vitamin B6; B12, vitamin B12; PA, pantothenic acid; FA, folic acid; BI, biotin.
the nitrogen source for 2-KLG production by *K. vulgare* and *B. megaterium* (Fig. 1). The coefficient of variation was 6% for mixed culture biomass, 8% for 2-KLG productivity, and 4% for rate of l-sorbose consumption, strongly demonstrating that the production of 2-KLG was significantly affected by the CSLP. However, the identities of CSLP ingredients that had major effects on 2-KLG production were unknown.

### 3.2. CSLP components affected by production batches

As mixed cultures of *K. vulgare* and *B. megaterium* cannot grow in a medium without CSLP (data not shown), CSLP not only provides the nitrogen source for cell growth but also offers essential nutrients. The quantities of individual CSLP components were determined for the 18 different batches. Amino acids were the crucial components, accounting for 30% of CSLP by dry weight. Glutamate (6476 mg kg\(^{-1}\)), alanine (3576 mg kg\(^{-1}\)), leucine (3453 mg kg\(^{-1}\)), and proline (3302 mg kg\(^{-1}\)) constituted 47.6% of the total amino acids. In addition, eight water-soluble vitamins were detected in CSLP, which did not contain vitamin C. The highest vitamin content was that of nicotinic acid, accounting for 80.9% of the total vitamins, followed by vitamin B6 (8.6%), and pantothenic acid (7.5%). Metal elements accounted for 10% of the CSLP dry weight, with K the most abundant (63.8% of all metal elements), followed by Mg (21.2%), P (8.19%), Na (4.4%), and Ca (1.8%).

As illustrated in Fig. 2, the quantities of 40 ingredients (17 amino acids, 9 vitamins, and 14 metal elements) in CSLP varied substantially among the different production batches, particularly those of metal elements, followed by amino acids, and then vitamins. The coefficient of variation for the amounts of arginine, tyrosine, methionine, cysteine, lysine, proline, Zn, Fe, Mn, Cu, K, Na, Mg, Ca, P, Cr, As, and Cd was higher than 20%, and the coefficient of variation for the concentrations of aspartate, histidine, alanine, valine, phenylalanine, vitamin B1, vitamin B2, nicotinic acid, vitamin B6, vitamin B12, and biotin was higher than 10%.

![Fig. 3. Grayscale map representing the linear correlation coefficients between CSLP main components and fermentation parameters. BKB, biomass of *K. vulgare* and *B. megaterium*; KLGP, 2-KLG productivity; RSC, rate of l-sorbose consumption.](image-url)
3.3. Relationships between CSLP components and 2-KLG production

Based on the above results, relationships between the quantities of CSLP ingredients and K. vulgare and B. megaterium growth, 2-KLG concentration, 2-KLG productivity, and L-sorbose consumption rate were determined (Fig. 3). The presence of glycine and threonine in CSLP significantly promoted the growth of K. vulgare and B. megaterium (r > 0.7), and the amounts of aspartate, serine, glutamate, histidine, nicotinic acid, biotin, and Cu also had positive effects on growth (0.5 < r < 0.7). The production of 2-KLG was predominantly determined by the amounts of serine, glycine, proline, nicotinic acid, and biotin (r > 0.7), with smaller positive linear correlations (0.5 < r < 0.7) between 2-KLG production and threonine, isoleucine, vitamin B1, Mn, and K (Fig. 3). CSLP serine, glycine, nicotinic acid (0.5 < r < 0.7), and proline (r > 0.7) had significant effects on the L-sorbose consumption rate (Fig. 3).

Linear correlation analysis with specific ingredients showed that the concentrations of individual amino acids were strongly correlated with each other, followed by metal elements and vitamins (Fig. 3). Of the amino acids, eight showed strong positive linear correlations (r > 0.7) between each other, and one showed a strong negative linear correlation (r < 0.7) with the others. Of the metal elements, two showed strongly positive linear correlations (r > 0.7) between each other, and none were strongly negatively correlated (r < -0.7). Only two strongly positive linear correlations (r > 0.7) were observed between individual vitamin concentrations. Strong linear correlations were also seen between amino acid concentrations and vitamin concentrations (two positive linear correlations), and between amino acid concentrations and metal element concentrations (six positive linear correlations and two negative linear correlations), whereas no significant linear correlations were detected between vitamin concentrations and metal element concentrations.

3.4. Effects of key factors on overproduction of 2-KLG by K. vulgare and B. megaterium

Based on the above results, a CDM with 40 ingredients was developed, replacing CSLP. The initial concentrations of the 40 ingredients were set equal to the corresponding initial concentrations in the medium with 5 g L\(^{-1}\) CSLP. The above results also suggested that glycine and threonine were the key components affecting mixed cell growth. Serine, glycine, proline, nicotinic acid, and biotin were the key components that affected 2-KLG production. The rate of L-sorbose consumption was affected by proline. As six key factors affected 2-KLG fermentation (serine, glycine, threonine, proline, nicotinic acid, and biotin), an L\(_{25}(5^5)\) orthogonal design experiment was employed to optimize the key factor levels, while maintaining the concentration of the other components (Table 2). The analyses, based on statistical calculations using the data in Table 3, are shown in Fig. 4.

Range analyses of the orthogonal design experiments indicate that: (1) glycine and serine were determinant to L-sorbose consumption and KGA synthesis (\(\alpha < 0.01\)); (2) threonine, proline, nicotinic acid, and biotin significantly promoted L-sorbose consumption and KGA synthesis (\(\alpha = 0.05\)); (3) threonine affected cell growth significantly (\(\alpha = 0.01\)). Serine, glycine, and threonine were found to be the important factors for 2-KLG production. At
0.28 g L\(^{-1}\) serine, 0.36 g L\(^{-1}\) glycine, and 0.18 g L\(^{-1}\) threonine, a high 2-KLG productivity was achieved. Serine, glycine, and threonine affected 2-KLG production by different mechanisms. Deficiencies of serine, glycine, and threonine have been suggested to affect nucleic acid biosynthesis because serine and glycine are donors of one-carbon units in one-carbon metabolism (Nikiforov et al., 2002;
Wang et al., 2009) and threonine can be converted into glycine and acetyl-CoA (Wang et al., 2009). Impairment of one-carbon unit pathways will weaken nucleic acid biosynthesis, protein biosynthesis, and methyl group biogenesis (Fox and Stover, 2008). These results are consistent with previous findings (Leduc et al., 2004). The addition of dihydrofolic acid, a precursor of the transmitter pathways will weaken nucleic acid biosynthesis, protein biosynthesis, and methyl group biogenesis (Fox and Stover, 2008). These results are consistent with previous findings (Leduc et al., 2004).

Glycine can also affect cell membrane permeability (Yang et al., 1998). A higher glycine concentration in the fermentation medium is believed to manipulate the metabolism by affecting membrane permeability. For B. megaterium, higher membrane permeability enhances the release into the fermentation broth of cytosolic components, which stimulate K. vulgare growth and 2-KLG production. However, for K. vulgare, membrane permeability may determine the secretion rate of 2-KLG. A number of studies have shown that proline is an osmotic protection agent (Aminard-Bretteville et al., 2003; Takagi, 2008). Previously, we found that osmotic stress continuously increases with the accumulation of 2-KLG. The mechanism by which proline enhances 2-KLG production may be by decreasing the destruction of microbial cells that occurs due to osmotic stress at high 2-KLG concentrations.

The results also demonstrate that nicotinic acid and biotin are key factors for 2-KLG production, biomass growth, and l-sorbose consumption rate. Nicotinic acid, the precursor of NAD+, is a cofactor in at least 727 biochemical reactions and of 456 enzymes in microbial metabolism (KEGG, updated on Mar 8, 2010). As 2-KLG production is an NAD+-related metabolic pathway, the presence of nicotinic acid provides NAD+ for 2-KLG synthesis. An obvious positive effect of biotin on biomass concentration and 2-KLG synthesis was also detected in this study. This is attributed to the involvement of biotin in the metabolism of unsaturated fatty acids, which are repressors of acetyl-CoA carboxylase (Birnbaum, 1970). In addition, biotin is also the cofactor for biotin-dependent carboxylases and has been demonstrated to function in cellular processes, including transcription and gene silencing (Healy et al., 2009).

The range analysis of the orthogonal design experiments gave the following two optimum concentrations for the key factors. For a high concentration of 2-KLG, the optimum medium composition was (Medium I, per L): 0.28 g serine, 0.36 g glycine, 0.18 g threonine, 0.56 g proline, 0.19 g nicotinic acid, 0.62 mg biotin. For a high yield of 2-KLG relative to l-sorbose, the optimum medium composition was (Medium II, per L): 0.28 g serine, 0.36 g glycine, 0.18 g threonine, 0.28 g proline, 0.19 g nicotinic acid, 0.62 mg biotin. A series of experiments were performed to test the optimized combinations. Both high 2-KLG concentration (58 g L⁻¹) and high yield (0.76 g g⁻¹) were achieved using Medium L in a 7-L jar fermentor, these six nutrients were sufficient for 2-KLG production (Fig. 5), and a higher 2-KLG concentration (58 g L⁻¹) and a higher yield (0.76 g g⁻¹) were achieved after 28 h culture using the combination of 0.28 g L⁻¹ serine, 0.36 g L⁻¹ glycine, 0.18 g L⁻¹ threonine, 0.28 g L⁻¹ proline, 0.19 g L⁻¹ nicotinic acid, and 0.62 mg L⁻¹ biotin (Fig. 6). The carbon balances before and after fermentation could be almost complete in SDM and CDM (Table 4). The carbon balance reduction in CDM may be due to increased respiration during the fermentation process, which would lead to more carbon dioxide being synthesized. The 2-KLG productivity (2.07 g L⁻¹ h⁻¹) and maximum biomass concentration (4.2 × 10⁹ cfu mL⁻¹) in this CDM were 99% and 230% higher, respectively, than in SDM (containing CSLP).

4. Conclusions

In this study, 40 major components of CSLP were analyzed to determine the key factors for 2-KLG fermentation, and a CDM was formulated based on the results. By supplementing serine, glycine, threonine, proline, nicotinic acid, and biotin at appropriate concentrations, a higher production efficiency of 2-KLG was achieved (58 g L⁻¹ 2-KLG concentration and 0.76 g g⁻¹ yield relative to l-sorbose). These results suggested that serine, glycine, threonine, proline, nicotinic acid, and biotin provided key nutrients, so a CDM was developed for 2-KLG production based on these findings.

Table 4

Fermentation properties of different media.

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<th>Parameters</th>
<th>SDM</th>
<th>CDM</th>
<th>Change (%)</th>
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<td>Fermentation time (h)</td>
<td>68</td>
<td>28</td>
<td>−58.8</td>
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<tr>
<td>L-sorbose consumption (g L⁻¹)</td>
<td>75.6</td>
<td>76.1</td>
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<td>Total biomass (g L⁻¹)</td>
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<td>4.37</td>
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<td>2-KLG concentration (g L⁻¹)</td>
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<td>2-KLG yield (g g⁻¹)</td>
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<td>0.76</td>
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<td>K. vulgare biomass yield (g g⁻¹)</td>
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<td>2-KLG productivity (g L⁻¹ h⁻¹)</td>
<td>1.04</td>
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<tr>
<td>Rate of L-sorbose consumption (g L⁻¹ h⁻¹)</td>
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<td>2.72</td>
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<td>Carbon balance (%)</td>
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<td>79</td>
<td>−11.2</td>
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Fig. 6. Time-course of batch fermentation for K. vulgare and B. megaterium. Initial l-sorbose consumption was 80 g L⁻¹. (●) 2-KLG production in CDM, (○) 2-KLG production in SDM, (□) l-sorbose consumption in CDM, (■) K. vulgare in CDM, (△) K. vulgare in SDM, (▲) B. megaterium in CDM, (△) B. megaterium in SDM.

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References


